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The Turnover of Cholic Acid in Man.*

Bile Acids and Steroids 51.

By

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Received 8 March 1957.

Problems related to the formation and excretion of bile acids in man have been studied by several authors (for reviews of earlier work see SOBOTKA 1937, JOSEPHSON 1941, TAYEAU 1949). Experimental difficulties such as the lack of reliable methods for the determination of the individual bile acids, have, however, made detailed quantitative studies impossible.

The estimates of the daily production of bile acids that have been published are usually based on determinations of the excretion of bile acids in bile fistulas in surgical cases or on the amount of the bile acids removed through a duodenal tube. By both these techniques, however, the normal enterohepatic circulation is broken. In animal experiments it has been repeatedly shown that the withdrawal of bile salts stimulates the liver to an increased synthesis (THOMPSON and VARS 1954, ERIKSSON 1956). We have tried to use a technique that would involve a minimum of disturbance of the normal physiological state. A tracer dose of ^{14}C-labelled cholic acid has been given and the specific activity of the circulating cholic acid determined in small samples removed through a duodenal tube at different times.

* A preliminary report of this work was read before the Norwegian Biochemical Society, June 1956.

Experimental.

Cholic acid-24-^{14}C had been prepared according to BERGSTRÖM, ROTTENBERG and VOLTZ (1953). Two different preparations with specific activities of 2.9 μC/mg and 1.005 μC/mg respectively were used. Stock solutions were prepared by dissolving the acid in acetone. One ml of the stock solution was added to about 2 g of inactive cholic acid in ethyl acetate. The cholic acid was crystallized (4.05×10^{-4} μC/mg and 8.75 μC/mg resp.) and used as a standard in the determination of radioactivity.

Administration of labelled acid. 2—4 ml of the stock solutions corresponding to 2.35—4.23 μC (2—5 mg) of cholic acid were evaporated to dryness and redissolved in a few drops of ethanol and then neutralized with 0.1 N sodium hydroxide. The solution was then diluted with water to 50 ml and given by mouth to voluntary male medical students (20—25 years old). The glass and the mouth were then rinsed with several portions of water. The subjects stayed on their ordinary diet and were told to avoid excess fatty food and alcohol.

Collection of bile samples. 20 hours after the administration of labelled acid the subjects swallowed a doudenal tube made from 2.1 mm polyvinyl tubing (Pharmaseal) (BLANKENHORN, HIRSCH and AHRENS Jr. 1955) loaded with 2 ml of mercury in a rubber balloon. After 3—5 hours the tube had usually passed through the pylorus as evidenced by the neutral reaction and green colour of the drainage. The subjects were then injected intravenously with 1.5—3 mg of a pancreozymine-cholecystokinine preparation (obtained from Professor E. JORPES, Stockholm) dissolved in water. In most cases a free flow of thick bile was then obtained from the tube within a few minutes. About 10 ml of this juice was collected and any excess given back through the tube.

Isolation of cholic acid. The bile was diluted with an equal volume of ethanol and the precipitate filtered off. The solution was evaporated to dryness on a water-bath and saponified with 25 ml of 2 N sodium hydroxide in a sealed glass-tube for 12—18 hours at 120° C. The free bile acids were then extracted with ether and cholic acid isolated by column chromatography on hydrophobic kieselguhr with the use of *iso*octanol/chloroform 1:1 as stationary phase and methanol/water 1:1 as moving phase [phase system C of NORMAN (1953)]. The fractions were titrated with 0.02 N sodium hydroxide. Those containing cholic acid were combined and extracted with ether after acidification. The cholic acid was crystallized from ethyl acetate. The yield varied between 5 and 50 mg depending on the concentration and amount of bile collected.

Determination of specific activity. The cholic acid was combusted and the specific activity of the carbon dioxide determined according to GLASCOCK (1954). The efficiency of the counting procedure was obtained from the combustion of a ^{14}C-polyacrylate of known strength (C. F. P. 1. The Radiochemical Centre, Amersham, England).

Results.

Evaluation of necessary assumptions for the determination of turnover rate.

1. *Bile acid pool.* The bile acids that predominantly occur in human bile are cholic, chenodeoxycholic and deoxycholic acids. These acids occur conjugated with glycine or taurine. The proportion between the acids varies between different individuals (WOOTTON 1953, ISAKSSON 1953—54, SJÖVALL 1957). The bile acids are practically completely confined to an anatomically restricted space consisting of the liver, bile ducts, gall-bladder, intestine and portal circulation. Under normal conditions the concentration of bile salts in the systemic circulation is negligible small (IRWIN, JOHNSTON and KOPALA 1944) and the bile acids are exclusively excreted in the feces (LINDSTEDT and NORMAN 1955). In healthy individuals this bile acid pool must be assumed to be in a steady state, *i. e.* the excreted bile acids are replaced by synthesis in the liver.

2. *Mixing within the pool.* The bile salts take part in an enterohepatic circulation as originally proposed by SCHIFF (1870). The time taken for a bile acid molecule to complete one enterohepatic circuit is not known. In animal experiments it has been shown, however, that an amount of labelled bile acid fed by mouth is rapidly absorbed and excreted in a bile fistula to the extent of 80—90 per cent within 2 hours (SJÖVALL and ÅKESSON 1955). It is also known that, when a meal reaches the duodenum, a large amount of bile acids is made available through the contraction of the gall-bladder (BORGSTRÖM, DAHLQVIST, LUNDH and SJÖVALL 1957). These facts indicate that a tracer dose of labelled cholic acid is rapidly mixed with the pool.

3. *Conjugation.* The labelled cholic acid was administered as its sodium salt. From preliminary work in humans and from many animal studies we know that the conjugation is complete when the acid has passed one time through the liver (NORMAN 1954).

4. *Interconversion of the bile acids into each other.* The interconversion of the different bile acids into each other in humans has not been much studied. LINDSTEDT and ZETTERSTRÖM (1957) fed cholic acid to a child and examined the bile soon afterwards. At this time no dihydroxycholanic acids were found. BERGSTRÖM and ZETTERSTRÖM (1957) in a similar experi-

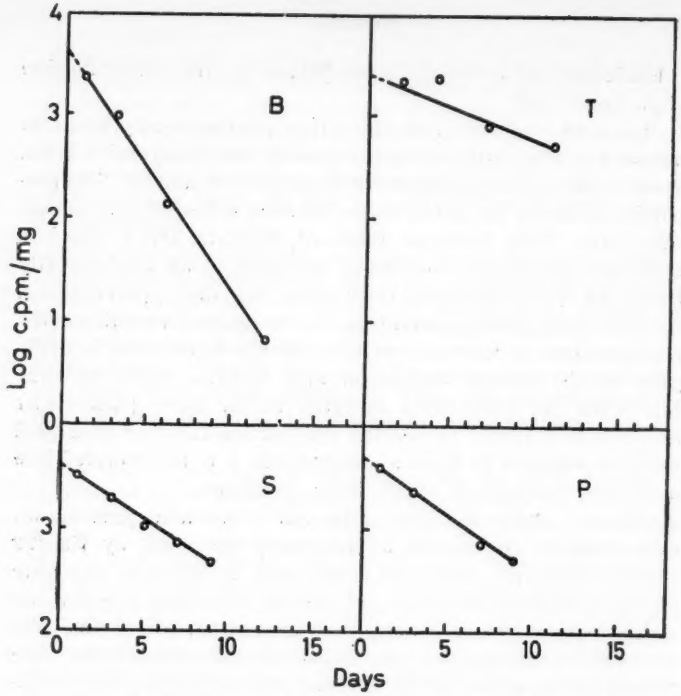

Fig. 1. Semilogarithmic plot of the specific activity of cholic acid in the pool.

ment found no evidence of formation of cholic acid from deoxycholic acid such as has been demonstrated in the rat by BERGSTRÖM, ROTTENBERG and SJÖVALL (1953). From now available evidence it, therefore, appears that the isotope does not re-enter the cholic acid pool once it has been removed from it.

Considering the facts discussed above it was reasonable to assume that the decay of specific activity of the cholic acid in the pool would follow first order kinetics. In this case

$$I_t = I_o\, e^{-kt}$$

where

I_t = specific activity of cholic acid in the pool at time t.
I_o = specific activity of cholic acid in the pool at zero time.
t = time in days.
k = "Elimination constant".

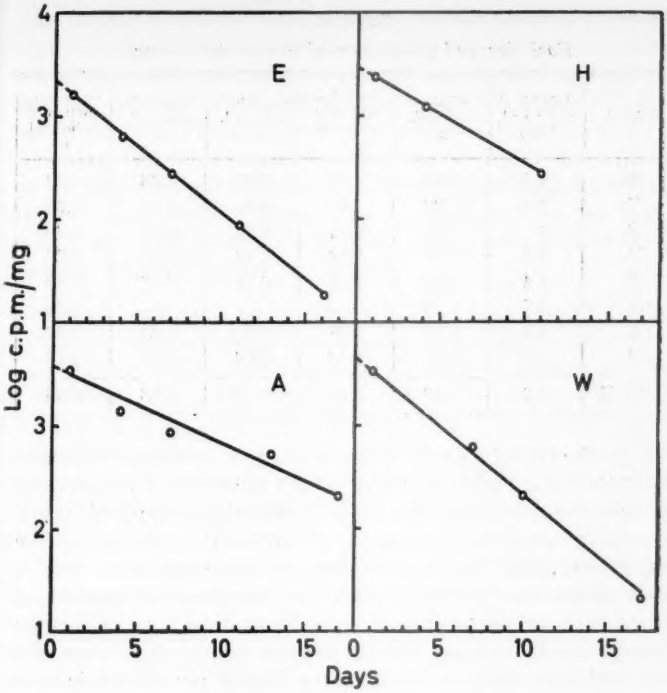

Fig. 2. Semilogarithmic plot of the specific activity of cholic acid in the pool.

and $$t_{1/2} = \frac{c \log 2}{k}$$

where

$t_{1/2}$ = half-life of cholic acid in days.

A plot of log I_t *versus* time would then give a straight line. As the first sample was taken one day after the administration of the tracer dose the value of I_0 was obtained by extrapolation to zero time. From the value of I_0 the amount of cholic acid in the miscible pool was calculated.

In fig. 1 and 2 a plot of log I_t versus time is shown. The linear fall is obvious. The values obtained from the graphs are tabulated in table 1.

Table 1.

Pool size and production of bile acids in man.

Subject	Half-life of cholic acid. Days	Cholic acid pool. g	Chenode-oxycholic acid pool. g	Deoxycholic acid pool. g	Total bile acid pool. g	Daily pro-duction of cholic acid. g
B.	1.2	0.50	0.79	0.51	1.88	0.33
T.	3.9	1.93	1.2	0.62	3.75	0.35
S.	3	1.29	1.70	0.92	3.91	0.30
P.	2.5	0.92	1.88	1.10	3.90	0.26
E.	2.4	2.29	1.88	0.78	4.97	0.69
H.	3.2	1.66	—	—	—	0.36
A.	4.2	1.27	1.25	0.80	3.32	0.27
W.	2.3	1.15	1.48	0.68	3.31	0.35
MEAN	2.8	1.38	1.45	0.77	3.58	0.36

With the use of a paper chromatographic technique (SJÖVALL 1956) SJÖVALL (1957) has determined the relative proportions between the three main bile acids in the subjects studied. When determinations were carried out at different times during the experiment only slight variations were encountered, and a mean value was therefore used. As the absolute amount of cholic acid in the pool had been determined by the isotope experiment, it was possible to use the quotients between the different bile acids to calculate the size of the total bile acid pool. The figures thus obtained are found in the table.

Discussion.

Choice of method. It has been repeatedly shown that the bile acids are extensively attacked by intestinal microorganisms (BERGSTRÖM and NORMAN 1953, LINDSTEDT and NORMAN 1955, EKDAHL and SJÖVALL 1955, GUSTAFSSON, BERGSTRÖM, LINDSTEDT and NORMAN 1957, LINDSTEDT 1957). No chemical methods are available that permit the determination of all bile acid derivatives in the feces. LINDSTEDT and NORMAN (1956) fed labelled bile acids to rats and estimated the half-life of the bile acids from the total excretion of isotope in the feces. This technique does not, however, give a figure for the size of the bile acid pool and would furthermore present technical difficulties in human experiments owing to the restricted amount of isotope that can be used.

As mentioned in the introduction it is not possible to reach a correct figure for the daily production of bile acids by measuring the excretion in a bile fistula or the recovery in duodenal drainage. In the present investigation only a few per cent of the total bile acids were withdrawn at intervals of 2—4 days, and this small amount would hardly upset the normal equilibrium. Cholecystokinine — a normal physiological stimulant — was used to bring about contraction of the gall-bladder at the sampling. The subjects had a meal a short time after the collection of the bile sample.

Half-life and pool size. The half-life of cholic acid varies between 1.2 and 4.2 days with a mean of 2.8 days. LINDSTEDT and NORMAN (1956) found the half-life of cholic acid in rats to be 2.3 days (1.2—3.1). In this animal chenodeoxycholic acid, litocholic and cholanic acids showed a half-life of the same order of magnitude. It must be pointed out, however, that the value obtained for cholic acid in this investigation cannot *a priori* be used for calculations of the turnover of chenodeoxy and deoxycholic acids in man.

When this work was started we believed that the three main bile acids in man were formed directly from cholesterol in the liver and that no interconversions between the acids took place. Recently, however, evidence has accumulated showing that deoxycholic acid is a "secondary" bile acid arising at least partially from the action of intestinal microorganisms on cholic acid (LINDSTEDT and SJÖVALL 1957, SJÖVALL 1957, LINDSTEDT 1957). If cholic acid is an obligatory precursor of deoxycholic acid the turnover of the cholic acid pool would be more rapid than that of the deoxycholic one in subjects where the bile contains more deoxycholic than cholic acid. In order to get a complete picture of the complex system it would be necessary to determine the turnover of the different bile acids in the same subject.

The size of the cholic acid pool showed large variations (0.58—2.29) giving a mean value of 1.39 g. It should be noted, however, that usually in the subjects with a small pool the half-life of the cholic acid was short. When the two other main bile acids were taken into account a value for the total bile acid pool of 3.58 g (1.88—4.97 g) was found. BERGSTRÖM and ERIKSSON (1957) found a value of 4.2—6.3 mg/100 g body weight for the bile acid pool in the rat, which is a figure of

the same order of magnitude as that found in the present work.

Relation to cholesterol metabolism. It is an established fact that the bile acids represent end-products of the cholesterol metabolism and there is no evidence against the assumption that cholesterol is an obligatory precursor of the bile acid. Our knowledge of the route by which the bile acids formed from cholesterol in man is, however, incomplete (ROSENFELD, HELLMAN and GALLAGHER 1955, SIPERSTEIN and MURRAY 1955).

From the data obtained in the present work we have calculated the daily formation of cholic acid to be 0.36 g (0.21—0.69). This corresponds to a daily degradation of 0.31 g of cholesterol. To this figure should be added the amount that is degraded to chenodeoxycholic acid which is probably of the same order of magnitude.

Summary.

Cholic acid-24-^{14}C has been given to male medical students. The specific activity of the circulating cholic acid has been followed. The half-life was 2.8 days and the size of the pool 3.58 g. From values of the quantitative composition of the bile the chenodeoxycholic and deoxycholic acid pools have been calculated.

I am much indebted to Professor E. JORPES and Dr. V. MUTT, Stockholm, for a generous supply of cholecystokinin.

The technical assistance of Mr. B. ÅKESSON and Mr. U. G. LINDEBERG is gratefully acknowledged.

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On the Effect of Iproniazid on the Histamine Sensitivity of the Unanaesthetized Guinea-pig.

By

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It has been observed that histaminase inhibitors augment some effects of histamine on unanaesthetized guinea-pigs (WESTLING 1956, 1957). This indicates that, under the prevailing experimental conditions, enzymatic inactivation by histaminase was one of the factors determining the sensitivity to histamine. Recent work on the urinary metabolites of ^{14}C-labelled histamine (SCHAYER 1952, 1953 a, and SCHAYER, KENNEDY and SMILEY 1953) has shown that in most species, including the guinea-pig, histamine is metabolized not only by histaminase (diamine oxidase) but also by another enzyme(s), provisionally called "histamine-metabolizing enzyme II". Histaminase inhibitors, *e. g.* aminoguanidine, had little effect on "enzyme II". On the other hand iproniazid was found to suppress the metabolism of histamine by "enzyme II" as well as by histaminase. In view of the previous observations with histaminase inhibitors (WESTLING 1956, 1957) it therefore seemed to be of interest to study the possible effect of iproniazid on the sensitivity of guinea-pigs to histamine.

The present paper contains some observations on the effect of iproniazid on the responses of guinea-pigs to histamine and some substances with similar actions. The experiments with iproniazid were done concomitantly with the mentioned studies on histaminase inhibitors (WESTLING 1956, 1957). In one series of experiments the respiratory symptoms elicited by histamine and 2-β-aminoethyl pyridine and the respiratory and cardiac

effects elicited by carbaminoylcholine were studied. In another series the response of the rectal temperature to histamine, 2-β-aminoethyl pyridine and 3-β-aminoethyl-1, 2, 4-triazole was measured. 2-β-aminoethyl pyridine and 3-β-aminoethyl-1, 2, 4-triazole are substances with histamine-like actions (for references see WESTLING 1957).

Experimental.

Animals. Male guinea-pigs were used. They were kept under the same conditions as the animals used in the previous experiments (WESTLING 1956, 1957). The animals used for studies on the respiratory effects had an initial body weight of 250—325 g; those used for experiments on the rectal temperature were slightly older and weighed 300—400 g. initially. *Substances*[1] *and injections.* The following substances were used: histamine acid phosphate (British Drug Houses), 2-β-aminoethyl pyridine dihydrochloride, 3-β-aminoethyl-1, 2, 4-triazole dihydrochloride, carbaminoylcholine chloride, aminoguanidine sulphate (Eastman Kodak) and iproniazid (1-isonicotinyl-2-isopropylhydrazine, "Marsilid" base, Roche). The substances were dissolved in 0.9 % saline solution. The doses of histamine, 2-β-aminoethyl pyridine, 3-β-aminoethyl-1, 2, 4-triazole and iproniazid are given in terms of the bases. Dosages for the other drugs refer to the salts mentioned. The concentrations of the solutions of histamine, 2-β-aminoethyl pyridine, 3-β-aminoethyl-1, 2, 4-triazole and carbaminoylcholine were chosen so that for the smallest dose given 0.1 ml of solution per 100 gram body weight was injected. Increase in dosage was effected by increasing the volume of solution injected. All substances were injected subcutaneously as described previously (WESTLING 1956, 1957).

Methods for measuring the response to histamine and other drugs. The duration of respiratory symptoms elicited by histamine and 2-β-aminoethyl pyridine and the duration of the respiratory and cardiac effects caused by an injection of carbaminoylcholine were estimated as described previously. The design of the experiments was also the same (WESTLING 1956). Each guinea-pig received saline or iproniazid subcutaneously 20—25 min. before the injection of histamine, 2-β-aminoethyl pyridine or carbaminoylcholine. Iproniazid and saline were usually given alternatively. In some experiments iproniazid and saline were prepared by an assistant and the nature of the solutions was unknown to the experimenter until after the experiment. The results in table 2 are expressed as the mean (±S.E.M.) of the differences observed in each animal between the values ob-

[1] 2-β-aminoethyl pyridine and 3-β-aminoethyl-1, 2, 4-triazole were made available through the courtesy of Dr. Reuben G. Jones, Eli Lilly Research Laboratories, Indianapolis, Indiana, U.S.A. Iproniazid was generously supplied by F. Hoffmann-La Roche & Co AG, Basel, Switzerland.

tained with and without iproniazid. The effect of 100 mg/kg of iproniazid was studied in the last experiment on each animal, since it was known that this dose of iproniazid affected the animal's general condition substantially.

The effects of histamine, 2-β-aminoethyl pyridine and 3-β-aminoethyl-1, 2, 4-triazole on the rectal temperature were measured as described (WESTLING 1957) except for the experiments in table 5, in which the effect of aminoguanidine and iproniazid on the response to histamine was studied. Only one experiment was made on each animal and the thermocouples were not kept permanently in the rectum but inserted every 10 minutes to a depth of 5 cm. The thermocouple gave a constant value 20 seconds after the insertion. The plan of the experiments on the rectal temperature was otherwise similar to that previously used (WESTLING 1957). The experiments in table 3 were performed after a 4×4 Latin square design. The results of table 4 are based on two experiments on each animal, one with iproniazid and one with saline.

The data were treated statistically as described (WESTLING 1956, 1957). Differences were regarded as significant when the probability (P) for them to be caused by chance was less than 0.05.

The experiments with observations on the external respiratory movements were performed in Oct. 1955—Febr. 1956. The experiments on the rectal temperature were made about one year later.

The effect of iproniazid alone on guinea-pigs.

The toxic effects of iproniazid on guinea-pigs have apparently not been studied. BENSON, STEFKO and ROE (1952) investigated the toxicity of iproniazid for mice, rabbits, rats and dogs. In mice the LD 50 for subcutaneously injected iproniazid was found to be 730 mg/kg. The mice injected with toxic doses of iproniazid developed clonic convulsions. In rats and rabbits iproniazid was found to be more toxic, the LD 50 being 383 and 125 mg/kg, respectively, after oral administration. In dogs prolonged administration of iproniazid in doses of 3.5 to 14 mg/kg led to pathologic changes in the spleen, bone marrow, liver and kidneys.

For the present study it was necessary to know whether an injection of iproniazid had any effect on the external respiratory movements of guinea-pigs. After a subcutaneous injection of 300 mg/kg of iproniazid a change in respiration was seen. The frequency of breathing increased and the external respiratory movements became larger. These changes lasted ½ to 1 hour. Doses of 100 mg/kg did not cause any observable changes in the appearance or frequency of the respiratory movements. Iproniazid was found to have delayed toxic effects. The six animals given 300 mg/kg of iproniazid lost much in weight and died after 2—7 days. The effect of smaller doses of iproniazid on the body weight is shown in table 1. The animals used for the experiments of table 1 were not used for potentiation experiments. It may be seen from table 1 that after doses of 10, 30 and 100 mg/kg of iproniazid a loss in body weight occurred. The greater part of the weight loss took place during the first 24 hours after the injection. The general condition of the animals given 100

Table 1.

Effect of a subcutaneous injection of iproniazid on the body weight of male guinea-pigs.

Dose of iproniazid (mg/kg)	3	10	30	100
No. of animals	3	8	12	11
Mean body weight at injection (grams).	313	313	300	316
Mean loss in body weight (grams).	0	6	16	46
Approximate time for reaching initial body weight (days).	—	1—2	4—5	6—7

mg/kg deteriorated substantially as well. The mechanism for this rapid decline in body weight after iproniazid is not known. An increase in output of urine or faeces could not be observed in animals kept in metabolic cages. The loss in body weight may possibly be due to diminished food intake.

Iproniazid in a dosage of 30 mg/kg given subcutaneously had no observable effect on the rectal temperature. 200 mg/kg of iproniazid, however, seemed to cause a slowly developing decrease in the rectal temperature. In one experiment in which the rectal temperature of 8 guinea-pigs was measured 4 were given 200 mg/kg of iproniazid and 4 served as controls, being injected with saline. At one hour after the injection the animals given iproniazid had a rectal temperature which was on the average 0.1° C lower than that of the controls. At two hours the difference was 0.26° C and at 4 hours it was still larger, namely 0.70° C. These experiments were performed in the same way as those in table 5; *i. e.* the thermocouples were not kept permanently in the rectum. The course of the rectal temperature in three other guinea-pigs, given 10 mg/kg of aminoguanidine in addition to 200 mg/kg of iproniazid, was similar to that in the animals given iproniazid alone.

Results.

1. *Observations on the respiratory movements.*

It was found that the respiratory effects of histamine lasted longer in animals that had been given iproniazid before the injection of histamine (table 2). The increase in duration was statistically significant after doses from 10 mg/kg upwards.

Table 2.

Duration of respiratory symptoms after subcutaneous injection of 1 mg/kg of histamine. Effect of a preceding injection of iproniazid. 16 male guinea-pigs. Mean values (±S.E.M.) for the increases in duration in each animal are given. The mean duration of symptoms after histamine, preceded by an injection of saline, was 67±7.9 min. (45 exp.). No guinea-pig died after histamine, preceded by saline.

Dose of iproniazid (mg/kg)	3	10	30	100
Mean increase in duration (min.)	12±5.0	28±5.1	42±4.7	51±5.8
No. of animals	7	7	7	7
No. of deaths	1	2	2	1

It should be noted that the 100 mg/kg dose is only 1/3 of the dosage of iproniazid found to produce effects on the respiration, when given alone.

The effect of iproniazid on the respiratory symptoms elicited by a subcutaneous injection of 13 mg/kg of 2-β-aminoethyl pyridine was studied in a group of 8 guinea-pigs. It was found that iproniazid had a pronounced potentiating effect. In 14 experiments in which saline was given to the animals before the pyridine compound the mean duration of respiratory symptoms was 69 ± 3.8 min. None of these experiments had a fatal outcome. In 8 experiments 30 mg/kg of iproniazid were given before 2-β-aminoethyl pyridine. 5 of these experiments had a fatal outcome. The death of the animals occurred under symptoms similar to those of a histamine shock. At autopsy the lungs were found to be emphysematous. The mean duration of respiratory symptoms in the surviving animals was 117±2.8 min. (3 exp.). In 7 experiments a smaller dose of iproniazid, 3 mg/kg, was injected. One of these animals died and in the others the mean duration of respiratory changes was 117±7.6 min. (6 exp.).

The effect of iproniazid on the duration of respiratory and cardiac effects of carbaminoylcholine (0.04 mg/kg) was studied in 8 guinea-pigs. After saline the mean duration of respiratory and cardiac effects was 37±2.7 min. and 48±5.5 min., respectively (16 exp.). Injection of iproniazid (30 mg/kg) instead of saline did not significantly change the duration of

Table 3.

Effect of iproniazid on the fall in rectal temperature induced by histamine. 4 guinea-pigs. Experiment performed after a 4×4 Latin square design. Mean values are given for the four observations on each treatment. S.D. for maximal fall 0.44; for duration 33.

Dose of histamine (mg/kg)	Preceding injection	Maximal fall (° C)	Duration (min.)
0.5	Saline	0.58	100
1.0	"	1.38	173
0.5	iproniazid 30 mg/kg	0.98	138
1.0	"	2.00	248

respiratory and cardiac effects, the mean change in duration being -1 ± 4.3 min. and $+3 \pm 6.8$ min., respectively.

2. *Observations on the rectal temperature.*

It was found that iproniazid in a dose of 30 mg/kg caused a slight enhancement of the effects of histamine on the rectal temperature. (Table 3.) The results were subjected to the analysis of variance, which showed that the increase in maximal fall after iproniazid was not significant. The increase in duration was significant ($P < 0.05$), however.

After 30 mg/kg of iproniazid the effects of 2-β-aminoethyl pyridine (8 mg/kg) on the rectal temperature were much augmented. This dose of iproniazid did not significantly modify the responses to 3-β-aminoethyl-1, 2, 4-triazole (table 4).

In one series of animals the action of aminoguanidine, iproniazid and a combination of aminoguanidine and iproniazid on the response to histamine was compared. The purpose was to see whether a large dose of iproniazid had a potentiating action by itself and, in particular, whether a combination of iproniazid with the histaminase inhibitor aminoguanidine would give a potentiation larger than that obtained with aminoguanidine alone. The dose of iproniazid chosen, 200 mg/kg, was known to have toxic effects, and, as mentioned earlier, it did by itself induce a slowly occurring fall in the rectal temperature. This dosage was used nevertheless since SCHAYER (1953 a) and SCHAYER *et al.* (1953) found that in cats and

Table 4.

Effect of a preceding injection of iproniazid on the changes in rectal temperature induced by 2-β-aminoethyl pyridine and 3-β-aminoethyl-1, 2, 4-triazole. Mean values for maximal fall (° C) and duration of response (min.) are given. The figures within parentheses denote no. of experiments. 4 experiments with 2-β-aminoethyl pyridine had a fatal outcome. In three of these experiments iproniazid had been given before.

Substance	Dose mg/kg	No. of animals	Responses with prior injection of saline	Responses with prior injection of iproniazid (30 mg/kg)
2-β-aminoethyl pyridine	8	11	1.20±0.14° C (9) 104±9 min. (9)	3.50±0.78° C (8) 256±28 min. (8)
3-β-aminoethyl--1, 2, 4-triazole	0.5	6	0.72±0.09° C (6) 93±13 min. (6)	0.77±0.12° C (6) 103±12 min. (6)

mice a similar dosage was necessary for almost complete inhibition of the "histamine-metabolizing enzyme II". Since the 200 mg/kg dose was known to impair the general condition of the animals for a long period of time it was decided to perform only one experiment on each guinea-pig. The animals in this series received one of four treatments 30 min. before the injection of 0.5 mg/kg of histamine. The treatments were saline, aminoguanidine 10 mg/kg, iproniazid 200 mg/kg and a combination of aminoguanidine (10 mg/kg) and iproniazid (200 mg/kg). The distribution of the animals between the four treatments was made at random. The effects of histamine on the rectal temperature in these four groups of guinea-pigs are shown in table 5. It may be seen that each one of the three inhibitor treatments increased the response to histamine. The differences in maximal fall and duration between the control group and each of the other three groups were all significant ($P<0.001$). The differences between the animals given iproniazid and those given aminoguanidine were not significant. The difference in response to histamine between the group given aminoguanidine and the group given iproniazid in addition to aminoguanidine was significant ($P<0.05$) as to maximal fall but not as to duration.

Concerning the results presented in table 5 it should be borne in mind that the dose of iproniazid used causes a gradual decline in the rectal temperature when given alone and that this makes the evaluation of the effect of histamine

Table 5.

Effect of aminoguanidine and iproniazid on the fall in rectal temperature elicited by histamine (0.5 mg/kg). 9 guinea-pigs in each group; 1 experiment on each guinea-pig. Mean values±S.E.M. for maximal fall in temperature and duration of response are given.

Preceding injection	Saline	Aminoguanidine 10 mg/kg	Iproniazid 200 mg/kg	Aminoguanidine 10 mg/kg + iproniazid 200 mg/kg
Maximal fall (° C)	0.70±0.10	1.42±0.07	1.72±0.19	1.74±0.09
Duration (min.)	97±10	161±12	167±12	191±13

hazardous. The fact that in many animals given iproniazid the rectal temperature, after the fall induced by histamine, did not fully return to the normal level but remained 0.2—0.4° C lower than before histamine underlines this point. Such was the case in six of the experiments with iproniazid and in four of those with a combination of iproniazid and aminoguanidine, but in none of the experiments with saline or aminoguanidine alone. In experiments in which the normal temperature was not restored the duration of the response to histamine was taken to be the time interval between the injection of histamine and the first value at the lowered level.

Discussion.

The results obtained may be summarized as follows: Smaller doses of iproniazid, up to 30 mg/kg, were found to augment histamine effects slightly. After this dose of iproniazid the responses to 2-β-aminoethyl pyridine were considerably increased, whereas the sensitivity to 3-β-aminoethyl triazole and carbaminoylcholine seemed to be unchanged. A large dose of iproniazid, 200 mg/kg, elicited a potentiation of the effects of histamine on the rectal temperature, which was slightly larger than the potentiation obtained by aminoguanidine. If iproniazid and aminoguanidine were given together the response to a subsequent injection of histamine was about the same as that after iproniazid alone and slightly larger than that after aminoguanidine.

A distinct augmentation of histamine effects was thus seen only after large doses of iproniazid, doses which had toxic

actions when given alone. A direct comparison of the potentiation with iproniazid and that with aminoguanidine was performed only in the experiments on the rectal temperature and it should be remembered that the dose of iproniazid used, 200 mg/kg, had a slight effect by itself on the rectal temperature. In spite of this the potentiation by iproniazid was not significantly larger than that obtained with aminoguanidine and the potentiating effect of a combination of aminoguanidine and iproniazid was only slightly larger than that of aminoguanidine alone.

It thus appears that both a histaminase inhibitor, aminoguanidine, and iproniazid are capable of potentiating histamine effects but that their actions are not additive. This result is in agreement with recent work (SCHAYER 1956, SCHAYER and KARJALA 1956) which has elucidated the nature of "histamine-metabolizing enzyme II". The authors mentioned found that this metabolic pathway consists of an enzyme, which methylates histamine to form 1-methyl-4-β-aminoethyl imidazole, and oxidative enzymes, which oxidize the methylated histamine to 1-methyl-imidazole-4-acetic acid. 1-isonicotinyl-2-isobutylhydrazine (IBINH) has the same inhibitory action as iproniazid on "histamine-metabolizing enzyme II", and it has been shown that IBINH does not inhibit the methylation of histamine, but blocks the oxidation of methylhistamine. Such an action would not materially increase the sensitivity to histamine, because in the pathway "histamine — methylhistamine — methylimidazole acetic acid" the main loss of biological activity would occur in the methylation. 1-methyl-4-β-aminoethyl imidazole has a biological activity, which is only 0.6 % of that of histamine, as estimated on guinea-pig ileum (LEE and JONES 1949).

The potentiation of histamine effects by iproniazid observed in the present experiments could be due to a direct inhibitory action on histaminase. Such an action has been observed in the living animal (SCHAYER 1953 a) and in vitro by *e. g.* LINDAHL, LINDELL, WESTLING and WHITE (1957).

However, iproniazid has complex pharmacological actions and great caution should be observed when interpreting the results presented. Iproniazid inhibits the metabolism of many drugs, thereby prolonging their action (FOUTS and BRODIE 1956). Iproniazid is also a strong inhibitor of amine oxidase

(*i. a.* ZELLER, BARSKY and BERMAN 1955, SCHAYER 1953 b, SCHAYER and SMILEY 1953, SCHAYER, WU, SMILEY and KOBAYASHI 1954.) It is known that histamine may release sympathicomimetic amines from the suprarenal medulla. Therefore, in a guinea-pig injected with iproniazid, more adrenaline may be available to counteract the effects of histamine. By the means of such interference the potentiation of histamine effects by iproniazid could be partly masked.

That iproniazid potentiated the effects of 2-β-aminoethyl pyridine even more than those of histamine was unexpected. The effects of another histamine-like substance, 3-β-aminoethyl-1, 2, 4-triazole, were not changed after iproniazid. It has been observed that 2-β-aminoethyl pyridine but not 3-β-aminoethyl-1, 2, 4-triazole is oxidized in vitro by extracts of guinea-pig liver (LINDELL and WESTLING 1957). The oxidation was inhibited by iproniazid and evidence was obtained which indicated that the enzyme responsible was amine oxidase. This may be the reason why iproniazid potentiated the effects of 2-β-aminoethyl pyridine as studied in the present investigation.

Summary.

1. Iproniazid augmented the effects of histamine on the respiratory movements and on the rectal temperature in unanaesthetized guinea-pigs.

2. Iproniazid caused a pronounced potentiation of the histamine-like effects of 2-β-aminoethyl pyridine but did not significantly change the responses to 3-β-aminoethyl-1, 2, 4-triazole and carbaminoylcholine.

3. The interpretation of the present findings is discussed.

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Secretory Potentials in the Sublingual Gland of the Cat.

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Electrophysiological experiments with microelectrodes were carried out on the submaxillary gland (LUNDBERG 1955). With intracellular recording it was found that with one type of gland cell the outer (basal) membrane was hyperpolarized during activation of the gland. The resting and active potentials over the inner (lumenwards) membrane could not be measured since attempts to record from the lumen of the acini were unsuccessful. From the direction of the external current flow it was, however, inferred that with activation a potential change took place also over the inner membrane of these cells, the lumen becoming positive to the cell interior.

It is of interest to study these secretory potentials since they may throw some light on the mechanism of secretion of salt and water. The work presented in this and in two subsequent papers has been carried out on the sublingual gland, in which it is possible to record not only intracellularly but also from the lumen of the gland cell tubules. As a preparation for investigation of gland cell function this gland offers the distinct advantage of having a poorly developed system of striated tubules.

The first section of this paper will present an account of the external electrogram recorded between one electrode on the free surface of the gland and another on the hilus, while the second section will concern microelectrode recordings from the cell interior and the lumen of gland cell tubules. Subsequent papers will deal with the mechanism of establishment of secretory potentials

and their significance for the understanding of the mechanism of secretion of salt and water. A preliminary report of some of the results has been given (LUNDBERG 1954).

Methods.

The cats were anaesthetized with chloralose 70 mg/kg intravenously. The dissection of the sublingual gland was often troublesome. The artery as well as the vein takes its origin from vessels within the submaxillary gland. For the experiments involving recording of external current flow all vessels except those to the sublingual gland were ligated and the submaxillary gland was removed. Thereafter the duct of the latter gland was removed from the surface of the sublingual gland, care being taken not to damage the parasympathetic nerve supply of this gland. The sublingual gland extends for 10—15 mm along the duct of the submaxillary gland. Along the sublingual gland duct there are small lobes of glandular tissue. For recording of the external electrogram these accessory lobes were removed after ligation of their ducts and the gland was placed on a small plastic tray, remaining in connection with the rest of the cat by the duct and nerve proximally and the artery and vein distally. In some cats it was impossible to dissect the gland so that it could be conveniently arranged for electrical recording. The sympathetic nerve fibres to the gland following the artery were usually destroyed during the dissection.

The technique for microelectrode recording was the same as described earlier (LUNDBERG 1955). The connective tissue appeared to be less dense than in the submaxillary gland and movements of the glands with activation were also less. Pulse-synchronous movements were often found and were in the later experiment sometimes eliminated after clamping of the ipsilateral common carotid artery. From the fact that the gland continued to function well it can be inferred that the blood supply through anastomosing vessels was sufficient. On the other hand the cells of the sublingual gland seemed to be more liable to mechanical damage than in the submaxillary gland and the connective tissue sheath must be removed with utmost care in order not to damage the cells.

The sublingual gland offers a distinct advantage because the lumen of the gland cell tubules can be dilated to allow recording. There are frequent reports in the literature that after stimulation the tubules are extended, and this became apparent in the present work after stimulation for a short period against obstructed or clamped duct. Quite often this dilatation occurred unintentionally during the experiment. The duct of the sublingual gland is often narrow, and, even if the cannula inserted into it was as thin-walled as possible, it often gave some degree of obstruction as judged from the dilatation of the tubules. With long-lasting stimulation against an occluded duct the dilatation of the lumen can become very marked so that the cells appear as a thin layer surrounding the lumen. The diameter of the lumen was kept

at 30 μ or less against an external diameter of the gland cell tubule of 50 μ. With more marked dilatation it was observed that the time-course of the secretory potential over the outer membrane changed so that, after having reached its maximum, it declined more than usually on continued stimulation. In order to view the tubules a dissecting microscope (Zeiss Opton) with a magnification of 100 or 160 was used. Indirect illumination was necessary to make the outline of the lumen visible. This was best achieved by focusing the light at some small distance from the actual site of inspection. Then the light was reflected by the underlying tissue and the outline of the lumen was made visible as well as the outer contour of the tubules. The occurrence of the dilatation and the degree of it was confirmed in histological sections.

Anatomy of the sublingual gland.

Throughout this work the gland used was the major sublingual gland situated in close apposition to the hilus of the submaxillary gland. The cells of the sublingual gland are arranged in a tubular fashion as contrasted to the submaxillary gland in which there are acini. With regard to the microscopical anatomy of the cells in the cat the views advocated in the literature are conflicting. Some early authors (Asp 1873, Ranvier 1886 and Illing 1904) claim that it is a mixed gland containing both mucous and serous cells. Other authors as Kolossow (1898) and Arima (1918) found only mucous cells in this gland.

Weissenfluh (1936) arrived at the conclusion that there is only one type of cell in this gland and called it "sublingualiszelle". This opinion was shared by Ziegler (1936). However, Järvi (1939) strongly opposes this interpretation and adheres to the classical concept of two types of cells, whereas Mühletahler (1942) agrees with the views presented by Weissenfluh and Ziegler. In the sublingual gland of the cat he describes many intermediate stages between mucous and serous cells and considers the sublingual gland cell a less specialized type of gland cell than those appearing in the submaxillary gland.

Histological work in connection with the present experiments has done little to clarify this question. It was regularly found that after stimulation all the cells appeared to be of a uniform type (rather resembling a serous cell) without a foamy cytoplasm and with rounded nuclei. Also in the resting gland these cells were by far the most common, but cells were encountered with the typical appearance of mucous cells *i. e.* flattened nuclei and foamy cytoplasm. As described by Mühletahler there were also intermediate stages of all different degrees.

The question of the cell types of the sublingual gland cannot be considered as finally settled. The electrophysiological observations described in this paper do not give evidence of two types of response as was found in the submaxillary gland. For the time being it may be assumed that with regard to the secretory mechanism dealt with in these papers there is only one type of cell.

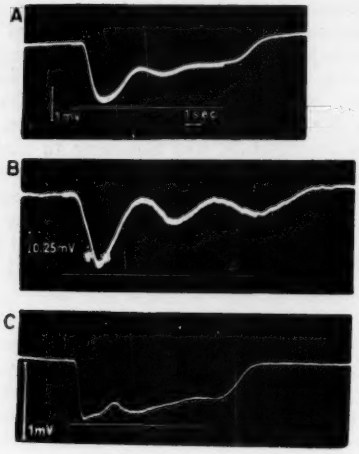

Fig. 1. Variation of the external chorda electrogram in the sublingual gland from three different cats.

The duct system of the sublingual gland has also received attention from the anatomists. MERKEL (1883) pointed out that this gland has not a well developed system of striated tubules; only isolated patches of rodded epithelium could be observed. Furthermore there are no intercalary ducts in this gland. It will appear that this less developed duct system in the sublingual gland offers advantages for the analysis of the mechanism of secretion.

Results.

1) External electrogram of the sublingual gland.

On recording between one electrode on the free surface of the gland and another on the hilus (or anywhere on the surrounding tissue) a potential change occurs when either of the secretory nerves are stimulated. The chorda electrogram is shown in Fig. 1. The records were obtained from three different glands on repetitive stimulation of the chorda at 25/sec. Downwards deflection of the beam denotes negativity of the electrode on hilus in relation to the electrode on surface of the gland. After a latency of about 1 sec. there is in each case a hilus-negative potential; the rate of establishment varies somewhat in the different cases. The potential reaches a maximum after 1—2 sec; thereafter it declines but to a different level in the three cases.

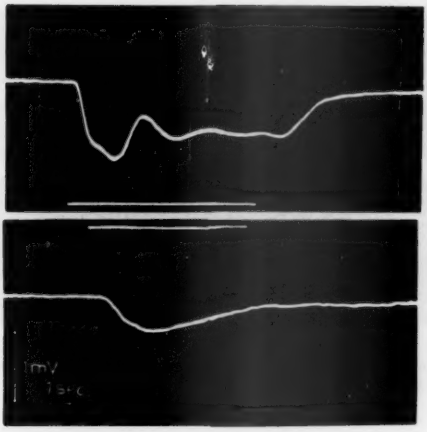

Fig. 2. Upper record shows the external chorda electrogram and lower record the external sympathetic electrogram.

In record C this decline of the potential during continued stimulation is quite small whereas in record B the potential decreases to zero thereafter to show an oscillatory course. This slow oscillation is found fairly often and is visible also in the other records. Record A is the type most commonly found. After having passed a maximum the potential decreases to about half the maximum size during continued stimulation of the chorda.

The records in Fig. 2 are both from the same gland. The lower records show the effect of repetitive stimulation of the sympathetic nerve at 15/sec. Also in this case a hilus-negative potential is established, which, however, decreases to zero with continued stimulation.

It is noteworthy that there is no slow phase in the sublingual electrogram. About one second after cessation of stimulation the potential starts to return to the resting level. The slow phase of the chorda as well as the sympathetic electrogram in the submaxillary gland (LUNDBERG 1955) was assumed to originate from the striated tubules, and as mentioned above the ducts of the sublingual gland have only a few cells with rodded epithelium.

It is thus clear that the external current flow initially has an opposite direction than in the submaxillary gland (BAYLISS and BRADFORD 1885, 1886, VAN HARREVELD 1930, LANGENSKIÖLD

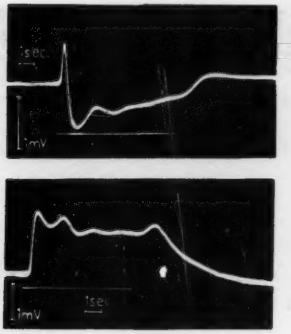

Fig. 3. Upper record shows the chorda electrogram from the combined submaxillary and sublingual glands. The lower record was taken from the submaxillary gland after excision of the sublingual gland.

1941, LUNDBERG 1955). However, the experiments on the sublingual gland were made on glands after an extensive dissection and it was felt necessary to do control experiments on glands that were not disturbed in this way. The upper record in Fig. 3 was obtained from the combined submaxillary and sublingual glands. After the initial hilus-positivity characteristic for the electrogram of the submaxillary gland the electrode on the hilus becomes negative in a way that is never found on recording from the submaxillary gland alone. Thereafter the sublingual gland was removed and the lower record shows the electrogram from the submaxillary gland. It is apparent from the records that the sublingual gland has contributed a hilus-negative potential as would be expected if the electrogram described in Figs. 1 and 2 were characteristic of the undissected gland.

2) Microelectrode recordings from the sublingual gland.

The gland cell tubules in the sublingual gland are more visible than the acini in the submaxillary gland, and in this work the microelectrodes were always inserted into an endpiece of a gland cell tubule under visual control. On insertion a potential jump occurs (internal negativity). Resting membrane potentials between 10 and 45 mV have been found and the mean value from 100 measurements in three experiments was 33 mV. With repetitive stimulation of the chorda nerve (25/sec.) this internal negativity increases. This is demonstrated in the lower record

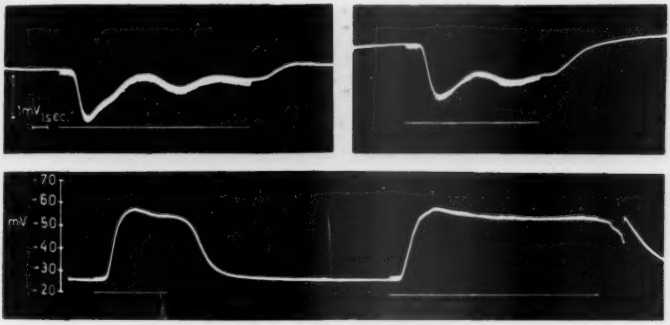

Fig. 4. Lower record shows intracellular recording from sublingual gland cell with two periods of stimulation of the chorda nerve. Upper records show external chorda electrogram from the same gland.

of Fig. 4. The latency is about 1 sec. and the hyperpolarization amounts to 30 mV but values between 20 and 35 mV were found. After having reached a maximum the potential usually declines 5 mV and thereafter remains on a plateau as long as stimulation is continued, in Fig. 4 for more than 13 sec. Quite often there is a potential jump during stimulation and the unit is lost; in Fig. 4 this occurs only after the long-lasting stimulation in the lower record. On the whole, however, movements of the gland are less troublesome than in the submaxillary gland, and the full course of the secretory potential resulting from 4—5 sec. stimulation was obtained more often than in the submaxillary gland.

The maximum rate of rise of the secretory potential was usually 30—40 mV/sec. but varied between 10 mV/sec. and 65 mV/sec.

The two upper records in Fig. 4 showing the external chorda electrogram from the same gland, were obtained at 5 minutes' interval and characteristically there are some differences in the later part of the electrogram. It is evident that both with regard to latency and rate of rise the intracellular recording and the external electrogram initially are similar. This strongly suggests that they have a common origin.

In the submaxillary gland two types of microelectrode responses were obtained on single shock stimulation of the chorda. In both cases a hyperpolarisation resulted; in the type I response it lasted for 1 sec. and in the type II response for about 10 sec. In the

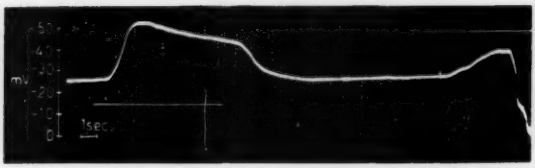

Fig. 5. Intracellular record from sublingual gland cell with secretory potentials resulting both on stimulation of the chorda (left) and the sympathetic nerve (right).

sublingual gland there is only rarely a potential change on single shock stimulation of the chorda, but, when it occurs, it lasts for about 3 sec., and the hyperpolarization has about the same rate of rise as when the chorda nerve is stimulated repetitively at 25/sec. If no potential change results with a single shock, a potential with this time-course can always be reproduced by a brief train of impulses in the chorda. This response is thus distinctly different from any of the two types found in the submaxillary gland.

The effect of sympathetic stimulation has not been systematically investigated for the reason that the dissection of the artery usually damages the sympathetic fibres. In a few cases such a damage was avoided, and it was then noted that sympathetic stimulation gives qualitatively the same effect, *i. e.* a hyperpolarization over the outer membrane, although usually only 10—20 mV. In one instance a hyperpolarization of 30 mV was found. Fig. 5 illustrates that also in the sublingual gland the cells may be innervated by both the sympathetic and the parasympathetic nerves. The unit was lost during stimulation of the sympathetic nerve.

The response described was the only type found in this gland. It should particularly be noted that no response was found in the depth of the gland similar to the type III response in the submaxillary gland (LUNDBERG 1955). This response was ascribed to the cells of the striated tubules and the failure to find the response in the sublingual gland should be expected from the fact that these cells are rare in the duct system of the sublingual gland (MERKEL 1883).

The measurements hitherto described are concerned with the resting and active potential over the outer membrane of the gland cells. If we assume that the external current flow is due to a po-

Fig. 6. To the left of the record there is an intracellular recording from a sublingual gland cell with secretory potential on stimulation of the chorda. The sudden jump of potential occurred when the tip of the microelectrode was inserted through inner membrane of the cell to record from the lumen the secretory potential produced by stimulation of the chorda.

tential difference between the external medium and the lumen of the gland cell tubules the hyperpolarization over the outer membrane may account for the initial hilus-negativity in the external electrogram, but since the potential in the later course of the electrogram declines more than does the hyperpolarization recorded over the outer membrane, we may expect a potential change with another time-course over the inner membrane.

In the sublingual gland the potential of the lumen may be measured directly after insertion of a microelectrode through the gland cells; prior to the insertion the lumen was dilated to have a diameter of maximally 30 μ (diameter of gland cell tubule 50 μ). The inner surface of the gland cells could be observed with the special illumination used, and, when the tip of the electrode penetrated through it, the potential jumped to become a few mV positive to the external medium. Thus the resting potential over the inner membrane is slightly larger than over the outer.

In Fig. 6 the microelectrode had been inserted through the outer membrane, and on repetitive stimulation of the chorda there is the usual secretory potential. Thereafter the electrode was slowly inserted further on. It was observed that the inner surface of the gland cell tubule dimpled slightly and then the sudden jump of potential seen in the record occurred. On stimulation of the chorda the microelectrode in this position again records a potential change in the negative direction which initially has very much the same time-course and magnitude as before penetration of the inner membrane. Consequently there is on stimulation initially very little change of potential over the inner membrane and the potential change recorded in the two sites occurs over the outer membrane.

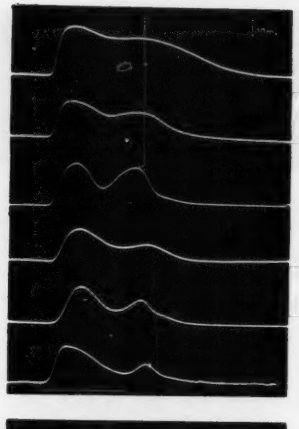

Fig. 7. Variation in time-course of secretory potentials recorded from lumen of six different gland cell tubules in one sublingual gland. The two lower records show the external chorda electrogram from the same gland and were obtained at an interval of 5 minutes.

If stimulation is continued there is, however, also a change of potential over the inner membrane. This may be observed in Fig. 6. After stimulation for 3 sec. the potential recorded from the lumen has decreased more than the potential recorded intracellularly. Furthermore it appears in Fig. 6 that after the end of stimulation the return of the potential recorded over the outer membrane is slower than the potential recorded over both membranes. This latter difference was not consistent; it may be due to a slower return of the potential over the outer membrane in the particular cell recorded from than in the average cell of this tubule.

Quite often the potential recorded from the lumen declines more with continued stimulation than does the potential recorded over the outer membrane. This is illustrated by the microelec-

trode records in Fig. 7, which were all obtained from a gland in which it was particularly easy to record from the lumen. Quite characteristically, after having reached a maximum, the potentials decline only to increase again with continued stimulation. Thus in many cases there is a potential change on activation also over the inner membrane of the gland cell, the lumen becoming more positive to the cell interior. The lower two records in Fig. 7 show the external chorda electrogram from the same gland. They were obtained at 5 minutes' interval and are not identical. It may be said that the time-course of the external electrogram represents an average of the potentials recorded from the different lumina, suggesting that the external current flow in this gland is due to the potential difference between the external medium and the lumina of the gland cell tubules.

As with the submaxillary gland (LUNDBERG 1955) the external electrogram and intracellular potentials have been measured during activation with injected acetylcholine and adrenaline. Close arterial injection of 1 μg acetylcholine gave hilus-negativity and with intracellular recording a transient hyperpolarization of 30—40 mV. Also with injection of adrenaline a hyperpolarization was found over the outer membrane.

Discussion.

The results of the potential measurements over the gland cell membranes are summarized in Fig. 8. The values given were actually obtained in an experiment in which recording was made successively intracellularly from a gland cell and from the lumen of the same tubule. A shows the resting potentials, B the potentials 2 sec. and C 4 sec. after the start of repetitive stimulation at 25/sec. of the chorda. Initially there is very little potential change over the inner gland cell membrane, whereas the outer is hyperpolarized by 25 mV. With continued stimulation there is a hyperpolarization also over the inner gland cell membrane amounting to 10 mV. Consequently the lumen becomes less negative to the external medium than in the early phase of stimulation.

The potential change over the outer membrane in sublingual gland cells has a slower time-course but is otherwise similar to the potential change found on secretion in gland cells (probably

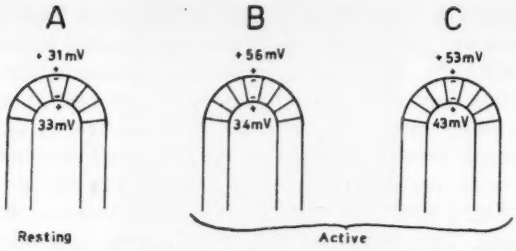

Fig. 8. Membrane potentials in sublingual gland cells. A in resting state, B 2 seconds and C 4 seconds after start of repetitive stimulation of the chorda nerve.

mucous) of the submaxillary gland giving the microelectrode response type I (LUNDBERG 1955). From the direction of the external current flow it was suggested that on activity a potential change occurred also over the inner membrane in these submaxillary gland cells, the lumen becoming positive to the gland cell interior. This potential change has now actually been found for the inner membrane in sublingual gland cells. There are, however, marked quantitative differences between the two types of gland cells. The slower time-course of the secretory potential over the outer membrane in the sublingual gland was referred to above; furthermore the assumed potential change over the inner membrane in the submaxillary gland must be as fast as over the outer membrane and it is probably larger than in the sublingual gland, since the sign of the potential change over the inner membrane decides the direction of the external current flow.

With regard to the mechanism of the establishment of these secretory potentials, it would from the above experiments only be possible to repeat the suggestions forwarded, when discussing the qualitatively similar potential changes in the submaxillary gland. The sublingual gland, however, offers better possibilities for a further analysis, and in subsequent papers it will be demonstrated that the secretory potential over the outer membrane is due to an inward active transport of anions.

Summary.

The sublingual gland of the cat has been used for electrophysiological measurement of the external current flow as well as intracellular potentials and the potential of the lumen of the gland cell tubules at rest and during activity.

The external chorda electrogram is hilus-negative. After passing through an initial maximum, it declines to about half its maximal size, often displaying slow oscillations. Also the external sympathetic electrogram is hilus-negative.

Only one type of microelectrode response was encountered in this gland. The resting potential over the outer gland cell membrane is about 33 mV (internal negativity). On stimulation of the chorda this potential difference increases to 50—65 mV, the latency of the potential change being about 1 second and the rate of rise 30—40 mV/sec. The latency as well as the initial time-course of this potential change is similar to that of the initial phase of the external chorda electrogram. Also with stimulation of the sympathetic nerve the potential difference over the outer membrane increases.

The resting potential over the inner gland cell membrane is a few mV larger than over the outer membrane (cell interior negative). On repetitive stimulation of the chorda there is initially little or no potential change, but with continued stimulation the potential difference over the inner membrane usually, but not always, increases by 10—15 mV.

The potential change recorded between the lumen and the external medium has a time-course similar to that of the external electrogram.

Acknowledgement.

The author is indebted to Dr. Dora Jacobsohn and to Dr. N. Å. Hillarp for histological work in connection with this investigation. The work was supported by a grant from the Swedish Medical Research Council. Technical assistance was given by Miss Ruth Araldsson.

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The Mechanism of Establishment of Secretory Potentials in Sublingual Gland Cells.

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A description of secretory potentials in the sublingual gland has been given in the preceding paper (LUNDBERG 1957a).

Experiments presented in the present paper are concerned with the mechanism of establishment of the secretory potential over the outer gland cell membrane. It will be demonstrated that it is due to an active transport of ions. Measurements of resistances of the gland cell membranes will be derived on the assumption that the gland cell tubule can be considered as a core conductor. The probable order of magnitude of the active transport represented by the secretory potential will be discussed in relation to measurements of electrolytes in the saliva.

An account of preliminary results was given in 1955 at the symposium in Paris on "The microphysiology of excitable tissues".

Methods.

In order to measure the potentials from the cell interior with simultaneous application of current double-barrelled electrodes were used. These were fabricated by melting two glass tubes together and this double-barrelled tube was thereafter pulled and filled in the usual manner. These electrodes could be made with a tip diameter of less than 0.5 μ and each channel had a minimal resistance of about 20 MΩ. The electrodes which were used had a common coupling resistance of 200 KΩ or less. This coupling resistance was checked both before and after the actual measurement had been made. Changes in the common coupling resistance during the course of the experiments were common, and all the measurements in which this occurred were dis-

carded. These double-barrelled electrodes were much more liable to break than the single ones as found by COOMBS, ECCLES and FATT (1955 a) with electrodes fabricated in another way.

For recording the same device was used as described earlier (LUNDBERG 1955). A switch in the input to the cathode follower permitting recording with current flow through either channel of the electrode. Recording was made against an earthed electrode in contact with the surface of the gland. The current generating device was free from earth and with one pole connected to a second electrode in contact with the surface of the gland close to the site of the microelectrode recording.

The strength of current was registered by recording the voltage drop over a calibrated resistance (1 MΩ in experiments with double-barrelled electrodes and 50 KΩ in the other experiments). The DC-amplifier used for recording the potential over this resistance was push-pull coupled and without grid leak in the input stage.

Results.

1. Secretory Potentials at Different Levels of the Membrane Potential.

It is natural to suspect that the potential change over the outer membrane is due to an active transport, but an alternative possibility would be that the permeability of the membrane is changed by the transmitter substance in such a way that anions enter or cations leave the cell over the outer membrane according to the electrochemical gradient for the respective ions in the resting state. If so, we would expect the size and the sign of the secretory potential to be dependent not only on the concentration difference for the respective ions, but also on the potential difference over the outer membrane. The secretory potential, normally a hyperpolarization of the membrane, would be diminished with increase of the membrane potential, absent at the equilibrium potential and show a reversed sign with still further increase of the membrane potential. However, if the secretory potential were due to an active transport of ions we can anticipate that its sign would not be changed even by a relatively large increase of membrane potential.

Fig. 1 illustrates an experiment with a double-barrelled electrode. All the records are from one cell. The record with a resting potential of 30 mV was obtained without current flow through the second channel. Thereafter a steady current was passed maintaining the membrane potential at 81 mV, but the secretory potential was virtually unaffected. Only when the membrane

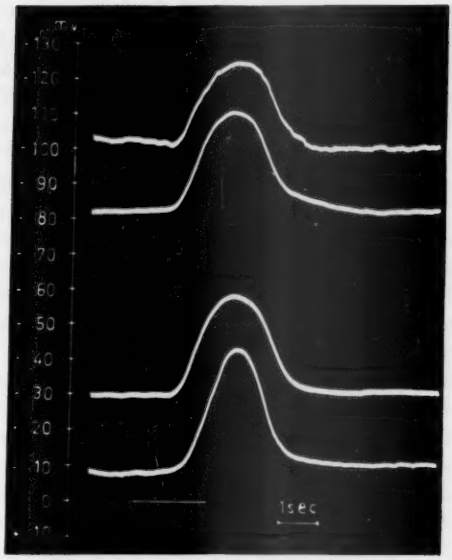

Fig. 1. Intracellular recording from sublingual gland cell with one channel of a double-barrelled electrode. Duration of repetitive stimulation of the chorda at 25/sec shown by white line. Record with a resting potential of 30 mV was obtained without current flow through the other channel and the remaining records with a current flow to change the resting potential as seen in the figure (potential due to common coupling resistance subtracted).

potential was increased to more than 100 mV was there a decrease of the secretory potential. In addition there were baseline irregularities at rest and during activity. This happened regularly when the potential by current flow was raised to this level and possibly indicates an instability or break-down of the membrane. With reversed current flow to lower the membrane potential there is also no change in the secretory potential resulting from an identical period of stimulation. The change in shape and magnitude observable in the lower record is not significant, since a later control record without current flow through the second channel gave a potential of similar size and time-course. On the whole the rate of rise and size of the potential may vary somewhat even in the same cell on repeated periods of stimulation, and small variations are not significant. The important fact is, however, that the secretory potential within wide limits is fairly independent of the level of potential.

The technique with double-barrelled electrodes greatly facilitates measurements from small cells, but obviously the results must be judged with caution. Apart from changes in the common coupling resistance due to deformation of the electrode there are other possibilities of error. The interpretation rests entirely on the assumption that the tip of the electrode really has penetrated the membrane. Even if the "outer" membrane has been penetrated properly as indicated by the sudden jump of the potential on insertion there are intracellular structures in gland cells, the transverse membranes (SJÖSTRAND, personal communication), which could be invaginated or in other ways give a hindrance to the current so that part of the recorded voltage drop may take place within the cell and not over the cell membrane proper. It was rather often found during the present experiments that with a current flow through one channel the potential recorded by the other fluctuated somewhat in an irregular fashion and with activation these fluctuations increased considerably. It is probable that in those cases the tip was in contact with some structure within the cell and that, with the mechanical changes always accompanying secretion, the contact changed and thereby also the voltage in immediate vicinity of the tip.

However, rather often the potential change on current flow was smooth and the secretory potential appeared without a background of noise and slow fluctuations of the potential. In such cases it was assumed that the electrode tip was not in contact with a structure that obstructed current flow, and consequently that the change of the intracellular potential was largely over the cell membrane proper.

The intracellular ionic composition of sublingual gland cells is not known. In the resting submaxillary gland WILLS and FENN (1938) (cf. MANERY, 1954) obtained the following figures in m equiv. per kg wet weight: potassium 85, chloride 54 and sodium 35. These values indicate that also in gland cells the resting membrane potential of about 30 mV is dependent on high intracellular potassium and low intracellular chloride. However, the equilibrium potential for either potassium or chloride could not be so high that the ionic flux represented by the secretory potential could be due to passive movement of ions across a membrane having a potential of more than 100 mV. Consequently it is concluded that the secretory potential over the outer membrane is caused by an active transport of ions.

2. Measurements of Resistance of Gland Cell Membranes.

a) *Measurements with double-barrelled electrodes.* In order to gain some information about the charge transferred over the outer membrane on activation it is of importance to measure the resistance of the membranes of the gland cells. The double-barrelled electrode technique is suitable for measurement of resistances between the cell interior and the external medium although such measurements obviously only give an overall value and do not supply information regarding the resistance of the different membranes of the cells.

Fig. 2 illustrates an experiment with a double-barrelled electrode. The current flow is recorded in each record by the upper beam. The upper record shows the hyperpolarization of the membrane that results from a gradually increasing current flow through the other channel of the electrode. With cessation of current flow the potential returns to the resting level and thereafter is shown the secretory potential resulting from chorda stimulation. Then the electrode was withdrawn in order to measure the common coupling resistance and thereafter reinserted into the same tubule for measurements from the lumen. In the lower record of Fig. 2 is shown the sudden changes of potential with penetration of the outer and inner membranes. Now a similar current flow gives only a very small potential change, which is of the same order as that due to the common coupling resistance. Therefore this technique cannot be used for measurement of the relatively low resistance between the lumen and the external medium. The mean value for the resistance (after subtraction of the common coupling resistance) in 50 cells was 2.0 MΩ with a standard deviation of 0.7. Obviously the same critical remarks are valid regarding these measurements as those made in the first section of this paper. This series of measurements is selected only those being chosen in which the potential change on insertion appeared suddenly and in which the potential change on current flow was stable.

Another attempt was made to measure this resistance by measuring the change in current flow through a single low-resistance electrode before and after introduction through the outer membrane. Quite often values of the same order of magnitude were found but on the whole the values were much more

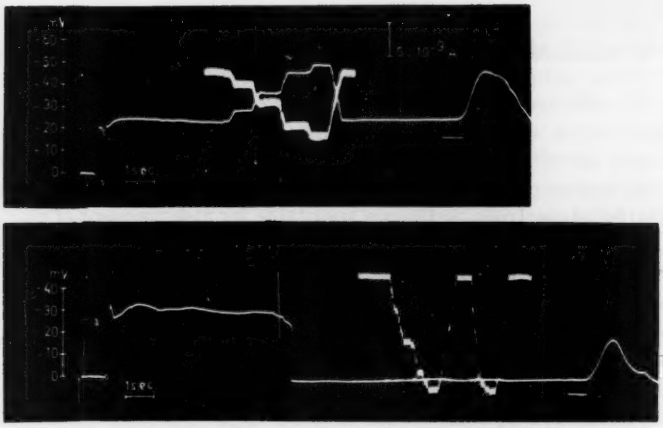

Fig. 2. Upper record shows intracellular recording from gland cell with one channel of a double-barrelled electrode. Sudden jump of potential in the beginning of the record during insertion of the tip through the outer cell membrane. Stepwise change of membrane potential caused by current flow through the other channel as indicated by second beam recording the potential drop of current over resistance.

The lower record was taken on reintroducing the electrode into the same tubule. After puncture of outer cell membrane causing the sudden shift of potential in the negative direction, the electrode was inserted through the cell to puncture the lumenwards membrane as indicated by the abrupt return of potential. Now with recording from lumen there was only a small effect of current flow through the other channel.

To the right in both records secretory potential from repetitive stimulation of the chorda during time indicated by white lines.

scattered and this technique is probably inadequate because of changes in the electrode resistance.

The resistance of the activated cell has also been investigated with the use of double-barrelled electrodes. Fig. 3 shows that during activation the potential change caused by the same pulse of current decreased by about 10 per cent.

b) *Core conductor properties of the gland cell tubules.* The further analysis of the electrical properties of the cell membranes is based on the assumption that the gland cell tubule may be considered as a core conductor. If we pass current between a microelectrode inserted into the lumen and an external electrode, it is assumed that the current flow through the tubular wall will take place as in a core conductor; the high resistance membrane will be the tubular wall and the saliva in the dilated lumen will constitute the low-resistance core. It is further assumed that current will not flow across the membrane between the cells *i. e.* that in

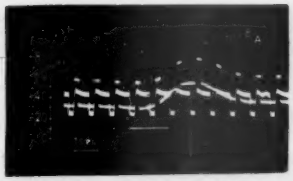

Fig. 3. Intracellular recording from sublingual gland cell with one channel of a double-barrelled electrode, with pulses of current passed through the other channel to demonstrate change in resistance during secretory potential. Intensity of current recorded by second beam as voltage drop over calibrated resistance. Beams displaced in time in order to allow better inspection of synchronous potential changes.

each cell the same amount of current flows through the inner and outer membrane. This implies that it is assumed that the gland cell tubule functions as a single core conductor and not as a double with two high resistance membranes. The intensity of current flow through the gland cells will decrease with increasing distance from the focus of the current flow. This decrease will be exponential in accordance with the cable equation $I_x = I_o e^{-x/\lambda}$ where I_o is the current intensity through the tabular wall at the focus, I_x x cm therefrom, and λ is the length constant of the cable (cf. KATZ, 1939; HODGKIN and RUSHTON, 1946). The length constant is given by the equation $\lambda = \sqrt{\frac{r_m}{r_i + r_o}}$ where r_m, r_i and r_o are respectively the resistances of unit length for the high resistance membrane, the core and the external medium.

The gland cell tubule is immersed in a large volume of external medium and r_o is so small in relation to r_i that it can be neglected, hence $\lambda = \sqrt{\frac{r_m}{r_i}}$. It has been pointed out by FATT and KATZ (1951) in their work on muscle fibres that $\frac{1}{2}\sqrt{r_m \cdot r_i}$ is the effective resistance between inside and outside. If the focus of current flow is at the end of a gland cell tubule we cannot use the term $\frac{1}{2}\sqrt{r_m \cdot r_i}$ directly. However, the effective resistance, r_e, is made up of two in parallel: the cylinder part of tubule and the end which is assumed to be a halfsphere, accordingly $\frac{1}{r_e} = \frac{1}{\sqrt{r_m \cdot r_i}} + \frac{1}{R_m / 2\pi a^2}$ when a is the radius of the dilated lumen

in cm and R_m is the specific resistance of the tubular wall in ohm cm^2 with respect to the area of the lumenwards membrane. Since we know the diameter of the lumen, R_m can be expressed as $r_m \cdot 2\pi a$, if we make the simplifying assumption that R_m is the same in the spherical end-piece of the tubule as in the cylindrical part. It is possible to obtain a value for r_i if we assume (cf. discussion) that the composition of the secretion in the lumen of the gland cell tubule is the same as in the saliva collected from the main duct, which is given in Table 2. This gives a value of about 50 ohm cm for the specific resistivity at 38° C and 7.1 MΩ for r_i with one cm as unit length and a diameter of the lumen of 30 μ.

It is therefore possible to derive r_m and hence also R_m from the measurement of the effective resistance between the lumen and the external medium. The ratio of the potential drop over the outer and inner membrane respectively when passing current between the lumen and the external medium can also be measured. However, on the basis of these measurements it would only be possible to derive the specific resistances if we knew the relationship between the in parallel resistances given by the gland cells and the intercellular spaces.

To obtain the data needed for the core conductor analysis the following experiments were performed. The tip of a microelectrode was inserted into the lumen through the gland cells of a selected gland cell tubule. During insertion the electrode was connected to the amplifier for measurement of the shift of potential occurring with penetration of the outer and inner membrane and then the chorda was stimulated for a brief period in order to establish that the usual secretory potential still could be recorded from the lumen. Thereafter the lead from the electrode was switched to allow current flow between this electrode and an external electrode, a large Ag-AgCl electrode, in broad contact with the gland, and the tip of a second electrode, connected to the amplifier was inserted through the outer membrane. Pulses of current were passed between the lumen and the external medium, and the recording electrode measures the drop of potential over the outer membrane that is caused by that fraction of the total current that passes through the cell recorded from. This can be observed in the upper record in Fig. 4, where it can also be observed that, with establishment of the usual secretory potential on stimulation of the chorda, the potential

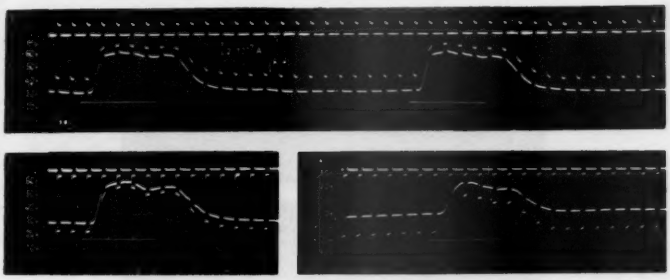

Fig. 4. Potential drop over gland cell membranes resulting from pulses of current passed between an external electrode and a microelectrode with the tip inserted into the lumen. Upper record and lower left record with intracellular recording from gland cells for measurement of change in resistance of the outer membrane during secretory potential. Lower record to the right with recording from the lumen to show change in resistance over inner and outer membrane during secretory potential. In all records the upper beam records the potential drop by current over calibrated resistance.

drop over the outer membrane decreases by about 40 per cent, showing that the resistance of this membrane is lower in the activated gland. When this measurement had been made, an attempt was made to insert the recording electrode through the inner membrane and repeat these measurements, or alternatively both electrodes were from the beginning inserted into the lumen. In Fig. 4 the lower record to the right (c) was taken after the tip of the recording electrode also had been inserted into the lumen. This record is from the same gland as the other records of Fig. 4, but not from the same gland cell tubule. It is evident that per unit current the potential drop caused by the brief pulse of current was now larger than with recording over the outer membrane. At rest the resistance is 90 KΩ and with activation it decreased to 75 KΩ. It was not easy to introduce two electrodes into the same lumen and only a total of 5 measurements were made. The resistances at rest were: 82, 90, 94, 104 and 160 KΩ, and the decrease in resistance with activation ranged from 12—28 per cent. The further calculations will be based on this small material, and it will be assumed that the effective resistance between the lumen and the external medium, r_e, in the average tubule is 100 KΩ at rest and 80 KΩ during activity.

Successful measurements over the outer and inner membranes on the same gland cell tubule were made only in two cases and one of them is illustrated in Fig. 5. Both the 'current electrode'

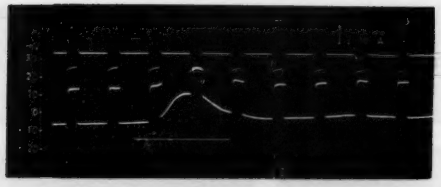

Fig. 5. Potential drop over gland cell membranes by pulses of current passed between an external electrode and a microelectrode with the tip inserted into the lumen. Both records were obtained from the same gland cell tubule; the upper with recording from the lumen, the lower with intracellular recording.

and the recording electrode were from the beginning inserted into the lumen and the upper record was obtained showing that the resistance was 104 KΩ at rest and 75 KΩ during activity. Thereafter the recording electrode was inserted further on for about 50 μ until it suddenly recorded a potential change to — 24 mV, presumably after having punctured the inner membrane of a gland cell. Expressed as that part of the effective resitance contributed by the outer membrane the values at rest and activity now were 55 and 29 KΩ respectively. In different observations this resistance at rest ranged from 20—65 KΩ and the decrease with activation from 15—60 per cent. In the upper record in Fig. 4 the resting resistance was 47 and the active 27 KΩ. The values 50 and 30 KΩ will be representative averages in successful recordings for the 'resistance contributed by the outer membranes' at rest and during activity.

For calculation of the resistance of the tubular wall from the effective resistance between the lumen and the external medium according to the formula given above we may use the value 100 KΩ at rest. With the diameter of the dilated lumen of 30 μ, this gives in the resting gland a value for r_m of 1700 Ω and for the transverse specific resistance of the tubular wall, R_m, of 16.2 ohm cm^2.

Similarly, in the active gland the value 80 KΩ will be used for the effective resistance between the lumen and the external medium. This gives us a value for R_m of 11.4 ohm cm^2.

c) *Evaluation of specific resistances for the outer and inner membranes.* When passing current between the lumen and the external medium the ratio of the potential change over the inner and outer membrane respectively was 1/1 in the resting state and 5/3 in the activated gland. The diameter of the tubule was 50 μ and of the lumen 30 μ. If we could assume that the intercellular spaces had infinitely high resistance it would be easy to derive the specific membrane resistances on the basis of the values for the specific resistance of the tubular wall given above as 16 and 11 ohm cm^2 int he resting respectively active state. For the outer membrane they would be 13 respectively 7 ohm cm^2 and for the inner membrane 8 respectively 7 ohm cm^2.

It is, however, not permissible to assume that the interspaces has an infinitely high resistance. We do not know the dimension of this space in the sublingual gland but we may provisionally assume that it is the same as in the pancreatic gland or 100 Å (SJÖSTRAND, personal communication). With the unlikely assumption that the tissue in the interspaces has the same specific resistivity as extracellular fluid, 50 ohm cm, it can be calculated that with the lumen dilated to 30 μ the resistance given by the intercellular spaces of one square cm tubular wall is about 15 ohm. This assumed value is incompatible with the values for the resistance of the tubular wall derived above, but it is evident that great attention has to be given to the shunting resistance offered by the interspaces, when attempting to evaluate the membrane resistances.

For the further discussion it is assumed that current, passed between the lumen and the external medium, flows in parallel through the interspaces and through the gland cells to give the total specific resistance for the tubular wall of 16.2 respectively 11.4 ohm cm^2. We may, then, for various assumed resistances of the intercellular spaces, calculate the gland cell resistance in the resting and active tubules, and from that also the specific resistance of the outer and inner cell membranes. In Table 1 A these calculations have been made assuming that the resistance of the intercellular spaces (column 1) is 1.5, 2, 3, 4 and 5 times the

minimal resistance of 15 ohm cm², which would result if the conductivity of the tissue in the interspaces were the same as in the extracellular fluid.

It is apparent that if the interspaces had a resistance of only three times the minimal the shunting effect of the current flow through the interspaces would not be very disturbing, with the resulting membrane resistances only about 50 per cent larger than with infinitely high resistance of the interspaces. Furthermore, with all assumed values for interspace resistance above three times the minimal the resistance of the outer membrane would decrease with activation by about 50 per cent whereas the resistance of the inner membrane would not change significantly. With the assumed lower values for the interspace resistance of 1.5 and 2 times the minimal one of 15 ohm cm², activation would give not only a larger decrease in the resistance of the outer membrane but also a considerable fall in resistance of the inner membrane. Now it should be remembered that initially with activation there is no potential change over the inner membrane and it would therefore be surprising if at that stage there was a resistance change in this membrane.

It is, however, possible to analyze this problem further on the basis of the values of resistances between the cell interior and the external medium reported under a) in this section. It was found that in the average cell this resistance was 2.0 MΩ at rest and 1.8 during activity. The resistance between the cell interior and the external medium is given by three in parallel: the outer membrane, the inner membrane and the membrane adjacent to neighbouring cells. It seems reasonable to assume that the resistance of the latter cell membrane does not change with activation. For each assumed value of interspace resistance in Table 1 A we can therefore examine if the derived values for membrane resistances in the resting and activated cell are compatible with the observed values of 2.0 respectively 1.8 MΩ for the resistance between the cell interior and the external medium. To make this comparison the following procedure was adapted. For each assumed value of interspace resistance the resistance of the inner and outer cell membranes have been calculated in the activated cell from the figures for the specific resistances presented in columns 5 and 7, Table 1 A (assuming hexagonal prim shape of the cell, cross dimension of 10 μ for the outer membrane, diameter of tubule 50 μ and the lumen dilated

Table 1.

A							B
1 Assumed interspace resistance per cm^2 tubular wall	2 Spec. resist. of resting gland cell in Ω cm^2	3 Spec. resist. of active gland cell in Ω cm^2	4 Spec. resist. of inner membrane at rest in Ω cm^2	5 Spec. resist. of inner membrane during act. in Ω cm^2	6 Spec. resist. of outer membrane at rest in Ω cm^2	7 Spec. resist. of outer membrane during act. in Ω cm^2	Spec. resist. of outer membrane at rest in Ω cm^2 derived as described in text
22.5	58	23.1	29	14.4	48.5	14.4	200
30	35.2	18.4	17.6	11.5	29.3	11.5	51
45	25.4	15.3	12.7	9.6	21.2	9.6	32
60	22.2	14.1	11.1	8.8	18.5	8.8	24
75	20.6	13.4	10.3	8.4	17.2	8.4	22
∞	16.2	11.4	8.1	7.1	13.5	7.1	14.5

A. Specific transverse resistances of gland cells, outer and inner cell membrane in the resting respectively activated state derived for various assumed values for the specific resistivity of the tissue in the intercellular spaces.

B. Specific resistance of the outer gland cell membrane needed to make to total resistance between the cell interior and the external medium 2 MΩ in the resting cell as compared to 1.8 MΩ in the activated cell. For the calculation it has been assumed that the resistance of the cell membrane adjacent to neighbouring cells does not change with activation. The resistance of this membrane was first derived in the activated cell from the values given for the specific resistance of the active outer and inner membrane in Table 1 A. This value was thereafter used with the value for the specific resistance of the resting inner membrane in A to calculate the specific resistance of the outer membrane of 2 MΩ between the cell interior and the external medium in the resting cell.

to 30 μ). From these data and the known value of the total resistance between the cell interior and the external medium of 1.8 MΩ the resistance of the membrane adjacent to other cells was found for each assumed value of interspace resistance. These values were then used with the corresponding values for the resistance of the inner membrane in the resting cell (derived from the specific resistances presented in column 4 Table 1 A) in order to calculate what in each case the resistance of the outer membrane must be to give the total resistance of 2.0 MΩ. Table 1 B shows the specific resistances for the outer membrane derived in this manner. If we compare these with the corresponding values in column 6 Table 1 A it is apparent that, only if the resistance of the interspace is 4 times or more the minimal possible, is there a reasonable agreement between these values. Hence it is concluded that the specific resistivity of the tissue in the intercellular spaces must be at least 4 times that of the extracellular fluid. Consequently the limiting upper value for the resistance of the

outer membrane is 18 ohm cm² at rest and 9 ohm cm² during activity. The corresponding values for the inner membrane are 11 and 9 ohm cm². It should not be concluded that there is a resistance change of the inner membrane with activation; the difference obtained is not significant.

The values for membrane resistance were derived on cells of the tubules with dilated lumen and hence with a very much larger area of the inner membrane than in the normal state. It is possible that with collapsed lumen, the inner membrane is 'folded' and that the total resistance of the inner membrane in one cell remains unchanged with dilatation. If so it would be rather misleading to give a value for the specific transverse resistance, which in the normal tubule with collapsed lumen would be considerably lower than the value of about 10 ohm cm² given above.

3. Relationship Between Electrolytes in the Secretion and the Secretory Potential Over the Outer Membrane.

The active ion transport over the outer membrane may be assumed to be an inward transport of anions. An outward transport of cations could give the same potential change, but such a movement would be opposite to the general movement of substances in secretion and is therefore an unlikely alternative. No other active process would necessarily have to be assumed in order to account for secretion of salt and water. The cations could follow passively due to the changed membrane potential and water would move osmotically. On the other hand it is of course possible that the inward movement of cations across the outer surface of the cell could be aided by an active transfer of cations; the increased potential difference across the outer cell membrane could result from a rate of active transfer of anions greater than that of cations. The possibility of a passive transfer of cations may be presented as an analogy to the frog skin where in the transfer of sodium chloride the movement of chloride is passive (cf. USSING, 1949c, 1954). In the frog skin this was proved by flux measurements and by the demonstration that the short circuit current equals the net sodium flux. It is not possible to imagine that such a strict comparison of electrical phenomena with ionic fluxes can be carried out in the salivary gland cells. However, with the frog skin as a model, we may find it useful to seek information that could give an indication of the order

of magnitude of the active transport over the outer membrane. For this purpose the ionic content of the saliva will be compared with the amount of current needed to equal the secretory potential from an external e. m. f.

The resistance of the fully activated outer membrane was found above to be 9 ohm cm². In order to equal the secretory potential in the upper record of Fig. 4, that results from stimulation of the chorda during 4.2 seconds, by current from an external e. m. f. over one square cm of membrane, the total transfer of charge needed would be 1.28×10^{-2} Coulomb or 0.13 μ equiv. of ions.

In order to get a figure for the electrolytes secreted by the gland, measurements have been made of the amount of saliva secreted by the sublingual gland and of the electrolyte content of this secretion.

Table 2 gives the concentration of K, Na and Cl in the saliva. Six samples of saliva from different animals were analyzed for sodium and chloride and three of them in addition for potassium. In order to collect the saliva the chorda was stimulated intermittently with 10 seconds pause after every period of stimulation for 10 seconds. WERTHER (1886) analysed the chloride concentration of the dog's sublingual saliva and found values between 120 mM and 198 mM in contrast to 26—57 mM for submaxillary gland saliva. The present analysis thus confirms his finding of a high chloride content. The ionic composition of an ultrafiltrate from the cat's blood plasma in mM is K: 5.8, Na: 158 and Cl: 124 (mean values from DAVSON, DUKE-ELDER and BENHAM (1936), D'SILVA (1936), KROGH (1946) and DAVSON, DUKE-ELDER and MAURICE (1949)). The water content of sublingual gland saliva is 98.72 per cent (WERTHER 1886) and consequently the figures in Table 2 need very little correction for comparison with the concentrations in blood plasma. It is noteworthy that the sublingual gland saliva is nearly isotonic with blood plasma, and further that the chloride concentration exceeds that in plasma. The composition is thus very different from that of the submaxillary gland saliva and this question will be discussed later in relation to the recent theories on the function of the striated ducts.

In order to obtain a figure for the amount of saliva the outflow of saliva from the duct of the whole gland was measured, and thereafter the gland was weighed. In four different glands stimu-

Table 2.

K, Na and Cl in Sublingual gland saliva in m mol/l.

	K	Na	Cl
1.....	8.2	144	143
2.....	10.7	151	163
3.....	9.5	156	166
4.....	—	159	161
5.....	—	170	172
6.....	—	156	163
Mean	9	156	161

lation for 4.2 seconds gave the following amounts of saliva expressed in ml/g glandular weight: 6.0×10^{-2}, 5.1×10^{-2}, 5.7×10^{-2} and 5.5×10^{-2}. These experiments were made on animals with wide ducts in order to have as small obstruction as possible, which is important since even a minimal amount of obstruction will cause dilatation of the tubules with the possibility of masked secretion. For comparison with the electrical data we need to express the amount of saliva with respect to the area of outer gland cell membrane. For this purpose it is assumed that the gland cells comprise 75 per cent of the weight of the gland and that they are arranged in tubules 0.5 mm long and with a diameter of 50 μ. The specific gravity of the gland determined according to CRINIS (1929) was found to be 1.06. The area of outer membrane per gram glandular weight will then be 5.7×10^3 cm^2. With an anionic content of 165 m equiv/l saliva the anions secreted on stimulation of the chorda for 4.2 sec by the number of gland cells having a total area of outer membrane of one cm^2 then comes to 0.018 μ equiv. Thus over any particular area of outer membrane the ionic flow needed to equal the secretory potential from an external e. m. f. is about 7 times larger than the anionic content of the saliva. However, it should be realized that, even if the active anionic transport were the sole active process responsible for secretion of salt and water, the anionic content of the saliva would not necessarily be a direct measure of this anionic flux. Passage of secretion from the cell interior into the lumen would be due to raised intracellular hydrostatic pressure and presumably some back filtration would take place

through the outer membrane. The anionic transport therefore may be higher than the figure obtained for the anionic content of the secretion but very probably not by a factor of more than two.

Now, if we could assume that the internal battery represented by the ionic transport and the current from an external e. m. f. have equal ability to create a potential difference over the outer membrane it would be possible to conclude that the active transport is large enough to account for the secretion of salt and water.

It remains to be discussed whether this assumption is justified. For simplicity we may assume that the D. C. conductivity of the membrane is made up by the partial conductances for Na^+, K^+ and Cl^- and that the anion actively transported is chloride. In case of current flow from an external e. m. f. the potential difference established by a certain current will depend on the total membrane resistance given by R_{Na^+}, R_{K^+} and R_{Cl^-} in parallel. With active transport through the same membrane of an equivalent number of ions the same potential would be established only if R_{Na^+}, R_{K^+} and R_{Cl^-} all were available as shunt resistances in parallel to the membrane battery responsible for the active transport of chloride ion, but not if R_{Cl^-} mainly was in series with the chloride pump.

The latter alternative may be raised because of the findings on the frog skin. On basis of experimental findings USSING and ZERAHN (1951) have proposed an equivalent circuit for this preparation with the sodium resistance in series with the sodium pump. LINDERHOLM (1952) has shown that the chloride conductivity plus the sodium conductivity equals the D. C. conductivity of the skin. Hence it has been concluded that the passive shunt resistance equals R_{Cl^-} (cf. USSING 1954). Accordingly we would in the frog skin expect that the current from an external e. m. f. and the sodium current resulting from the pump would give the same potential difference across the skin only if R_{Na^+} and R_{Cl^-} were equal. If the shunt resistance were larger than the series resistance the active transport of sodium needed to establish a certain potential would be smaller than the ionic current from an external e. m. f. Similarly it is evident that if in the activated outer gland cell membrane, R_{Cl^-} were largely in series with the chloride pump it would not be permissible to equalize the ability of an external e. m. f. and the internal battery to establish a potential difference over the outer cell membrane.

It is, however, probably unwise to carry the analogy with the frog skin too far. Compared with a cell membrane the frog skin is a complex structure and presumably the resistance across the skin is only partly in the actual membrane responsible for the active transport. It would therefore be reasonable to expect an appreciable resistance in series with the pump, whereas this must not necessarily be the case with respect to an ion pump in a cell membrane.

Another difference between the two preparations stems from the fact that in the frog skin the actively transported sodium ion is larger than the passive ion. With the outer gland cell membrane the actively transported chloride ion is smaller than the main passive ion, sodium. According to the widely accepted concept of a lipoid pore membrane, as proposed by COLLANDER (1937) inorganic ion penetrate only in so far as the ion diameter is smaller than the pore diameter. With this concept we would expect that the pores through which sodium ion pass passively are available also to chloride ion and consequently that R_{Cl^-} acts as a passive shunt resistance.

It is, however, known that passive ion movements caused by a difference in electrochemical potential may depend on other than pure physical mechanisms such as pore size. The specific permeability increase for sodium associated with the nerve impulses is assumed to be due to a chemical process, the sodium carrier mechanism (HODGKIN 1951, HODGKIN and HUXLEY 1952, HUXLEY 1954, HODGKIN and KEYNES 1955). This mechanism is, however, associated with a highly specialized function in nervous tissue. Even in nervous tissue other types of passive ionic fluxes such as occurs when nerve cell membranes are acted upon by excitatory or inhibitory transmittors are explicable on the basis of pore size theory (COOMBS, ECCLES and FATT 1955 b, c). This is particularly well illustrated by the dependence of the inhibitory postsynaptic potential on the electrochemical potential differences only for ions under a certain size (COOMBS, ECCLES and FATT 1955 b).

Presumably R_{Cl^-} could be in series with the chloride pump only if the permeabilities in the membrane were chemical (as opposed to a physical mechanism depending on pore size). On the basis of the pore size hypothesis it would seem that there is some justification for the idea that the order of magnitude of the active transport can be appreciated from the transfer of

charge that is needed from an external e. m. f., to equal the secretory potential. This would hold true even if the permeabilities were 'chemical' as long as R_{Cl^-} were not in series with the pump. The approximate nature of the comparison made is nevertheless well worth emphasizing, the drag effect of water has been neglected and the recent disclosure by HODGKIN and KEYNES (1955) of the complex nature of passive movement of potassium ion through the membranes of giant nerve fibres certainly give reason for carefulness in the present case. These experiments can therefore not give conclusive evidence compared to the results obtained with direct measurement of ionic fluxes, but undoubtedly this comparison has indicative value in so far as the results show that to give the secretory potential of the recorded magnitude over the outer membrane the active anionic transport must be considerable, and may very well be sufficiently large to account for the electrolytes in the saliva.

Discussion.

This investigation has been concerned mainly with the secretory potential over the outer membrane, which is the dominating electrical event in the gland cells on activation. With the conclusion that this secretory potential is due to an active transport of anions it is not excluded that an active transport of cations also may take place in this membrane. The sign of the secretory potential could be a consequence of a faster active transfer of anions than of cations. With the isolated frog skin USSING (1949 a, b), KOEFOED-JOHNSEN, LEVI and USSING (1952) and USSING and ZERAHN (1951) were able to prove that the potential over the skin is caused by the active transport of sodium ion and that the passage of chloride is passive. This was achieved through measurements of the ionic fluxes by tracer technique and also by the demonstration that the short circuit current equals the net flux of sodium.

With the outer salivary gland membrane it is clearly impossible either to short-circuit the membrane or to measure the ionic fluxes over this membrane, so it has been necessary to rely on more indirect methods. Values for the resistances of the gland cell membranes have been derived, and on this basis a comparison has been made per unit area between the anionic content in the secretion and the transfer of charge from an external e. m. f. that is needed to equal the secretory potential. Since

the latter figure is in excess of the former it is suggested that the anionic transport that is responsible for the sign of secretory potential is large enough to account for secretion of salt and water. It has already been pointed out that this suggestion is based on the assumption that current from an external e. m. f. and the anionic current of the active transport have approximately equal ability to create a potential difference over the outer membrane. The figures found make it unlikely that any large inward active cationic transport is concealed by the active transport of anions. Further evidence that the inward active anionic transport over the outer membrane is the major active process responsible for secretion of salt and water will be given in a paper on secretion and secretory potentials in glands perfused with chloride-free saline (LUNDBERG 1957 b).

With the composition of the saliva in Table 2 the anions transported must be largely (or exclusively) chloride. An active transport of chloride has been demonstrated in other tissue containing gland cells. When the frog skin is stimulated by adrenaline an outwards active transport of chloride ion starts and this transport was assumed to be performed by the skin glands (KOEFOED-JOHNSEN, USSING and ZERAHN 1952). An active transport of chloride ions is also performed by the isolated frog gastric mucosa (HOGBEN 1952).

In the sublingual gland the other events leading to secretion of salt and water may be secondary to the active transport of chloride. The entry of chloride ions make the cell interior more negative and with this potential difference the cations follow passively. These cations must be largely sodium. The ratio K/Na in the sublingual gland saliva is somewhat larger than in plasma but it cannot be concluded that potassium is preferred (due possibly to a lower permeability than for sodium) because intermittent stimulation was used for collection of the saliva and part of the potassium in the saliva may have an intracellular origin (cf. BURGEN, 1956). The entry of ions gives a high intracellular osmotic pressure causing the water to enter and the raised intracellular hydrostatic pressure thus resulting would give filtration through the inner membrane (as well as through the outer). However, on secretion there is a small potential change also over the inner membrane of the gland cell. If this potential represented an active transport, the secretory efficiency might be increased because back-filtration over the outer membrane would decrease.

One important assumption for the present work has been that the composition of the saliva is not changed to any considerable extent during the flow through the ducts. It is known that the saliva from some glands is markedly hypotonic and recent experiments (SCHWARTZ, THAYSEN and DOLE 1954, THAYSEN, THORN and SCHWARTZ 1954, THAYSEN and THORN 1954 and THAYSEN 1955) have led to the theory of reabsorption of sodium chloride in the striated tubules. There are only few striated cells in the duct system of the sublingual gland (MERKEL 1883) and the finding that the sublingual gland saliva is approximately isotonic with blood plasma is in agreement with the findings by THAYSEN and co-workers on other glands without striated tubules. This isotonicity of the secretion in glands without reabsorptive ducts is noteworthy, because the hypothesis of secretion forwarded would be incompatible with a hypotonic "primary secretion". It is also of interest that the sublingual gland has no intercalary ducts, the function of which we know nothing about. We can therefore with some confidence assume that the composition of the 'primary saliva' as secreted by the cells is very similar to what is found for the saliva collected from the main duct.

Since the secretory potential over the outer membrane is a measure of the activation of the gland it may be used as indicator for the examination of problems concerning gland cell innervation. The question of gland cell innervation has been dealt with in a paper on the electrophysiology of the submaxillary gland (LUNDBERG 1955). In all likelihood the secretory potential in the cell giving response type I in this gland is of the same nature as the one found in the sublingual gland. The present findings on the mechanism of establishment of the secretory potentials therefore serves to emphasize the validity of the conclusion drawn from the experiments on the submaxillary gland. The sublingual gland is a less favourable preparation for investigation of innervation problems because of the necessity to work with potentials resulting from single shock stimulation. Such a response is encountered only rarely in the sublingual gland cells, but when found it has been observed that the secretory potential can be graded with the strength of the stimulus to the chorda, *i. e.* that there is convergence of para-sympathetic neurones onto one cell as was concluded to be the case with submaxillary gland cells.

It is of interest that the process of activation with junctional transmission or injection of the transmitter substance appears to be different than in other effectors investigated. There is no indication that the gland cell membrane is electrically excitable nor that the gland cell excitation is a regenerative process comparable to excitation in nerve fibres (cf. HODGKIN 1951). The finding that the magnitude of the secretory potential can be graded with the intensity of chorda stimulation as well as with the amount of injected acetylcholine can only mean that the intensity of the active transport started by the transmitter up to a certain level is dependent on the concentration of the transmitter substance.

Summary.

An attempt has been made to analyse the mechanism of establishment of the secretory potential over the outer gland cell membrane in the sublingual gland of the cat, as well as its importance for the secretion of salt and water.

Double-barrelled microelectrodes were inserted into gland cells permitting recording of the intracellular potential with current flow through one channel to change the membrane potential. The secretory potential over the outer membrane could still be evoked when the membrane potential had been raised to more than 100 mV. It is concluded that this secretory potential is due to an active transport of ions over the outer membrane and assumed to be an inward transport of anions.

The resistance between the cell interior and the external medium measured with this technique was about 2 MΩ in the resting state and 1.8 MΩ during activation. Measurement has also been made of the resistance between the external medium and the lumen of the gland cell tubule and of the ratio of the resistances offered by the inner and outer gland cell membranes to a current passed between the external medium and the lumen. On the basis of these measurements and the assumption that the gland cell tubule can be considered as a core conductor the specific resistances of the outer and inner gland cell membrane were derived to 18 respectively 11 ohm cm^2 in the resting cell. With activation there was no significant change in the resistance of the inner cell membrane, whereas the resistance of the outer membrane decreased by about 50 per cent.

The anionic content of the saliva secreted by a number of gland cells with a certain area of outer membrane has been compared with the amount of current needed from an external battery to equal the secretory potential over this area of outer gland cell membrane. The probable order of magnitude of the active anionic transport is discussed in view of these findings.

The hypothesis is forwarded that secretion of salt and water largely is caused by active transport of anions (mainly or exclusively chloride) over the outer gland cell membrane.

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Effect of Tributyrin on Circulation and Respiration.

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In attempts to produce fat emulsions for intravenous nutrition, the question arose of which neutral fats were most suitable for this purpose from the physical point of view. It was considered motivated for several reasons to perform experiments with low-molecular neutral fats. In tests with a 10 per cent emulsion of tributyrin, injected intravenously into the rabbit, symptoms nevertheless appeared that had not been observed after far larger doses of *e. g.*, olive oil or cottonseed oil emulsions. The animals lay on their side, and relatively lengthy apnoea appeared, followed by violent hyperpnoea. In addition, salivation, miosis and bradycardia were present. In view of these observations, it was considered advisable to make a closer study of the pharmacological effects of tributyrin. In the animal experiments described in the following, the chief factors studied were the toxicity of tributyrin, and its effects on circulation and respiration.

Experimental.

Tributyrin. In order to rule out any impurities in the tributyrin, it was redistilled *in vacuo*. Since tributyrin is poorly soluble in water, it was used in the form of an emulsion of the following composition:

Tributyrin	10 g
Soy-bean phosphatides	0.75 g
Demal 14	1 g
Glucose	5 g
Distilled water	to 100 ml

The soy-bean phosphatides and Demal 14[1] (polyglycerol monooleate) were used as emulsifying and stabilizing agents.

The *soy-bean phosphatides* were purified as follows: 200 g of "lecithin"[2] were dissolved in 800 ml of ether and filtered. The phosphatides were then precipitated by addition of 6 parts of acetone. The precipitate was separated off by centrifugation. This precipitation procedure was repeated three times. After the last centrifugation, the precipitate was dried *in vacuo*. For preparation of the emulsion, all the ingredients were mixed in a Warring blender after heating to 80° C; the mixture was then homogenized in a Logeman homogenizer for 5 minutes per 100 ml mixture. The emulsion prepared in this way is stable for at least 2 to 3 months if kept in a refrigerator. Microscopic examination showed it to be homogeneous. The diameter of the fat particles was less than 1 μ.

For control experiments, the same emulsion was prepared without tributyrin. In order to ascertain the eventual pharmacological effect of one of the hydrolysis products of tributyrin, 1 per cent of butyric acid was added.

In some cases, the 10 per cent emulsion was diluted with a 5 per cent glucose solution.

The effect on *circulation* and *respiration* was tested in cats, anesthetized with diallymal ("Dial", Ciba), 25—50 mg/kg of body weight. The blood pressure was recorded on a drum via a mercury manometer connected to a cannula in the femoral artery. The respiration was recorded through a Marey capsule connected to a lateral tube in the tracheal tube.

Determination of the flow in the abdominal aorta was performed with a special apparatus (WRETLIND 1957). This permits direct, exact recording of the blood flow per time unit (0.5, 1, 2 or 3 sec.). For this purpose, the blood is diverted to the apparatus for a certain time. In the apparatus a rubber membrane is displaced. The displacement of the membrane is then recorded on the drum.

The effect of tributyrin on the action of the heart was studied by means of STARLING's heart-lung preparation. The cat was used as the experimental animal, and the preparation carried out according to THER (1949). The apparatus for extracorporeal circulation is a modification of an earlier arrangement according to GAUTRELET (1932), KRAYER (1932) and others. Its construction is shown in Fig. 1. The flow was measured with a flowmeter according to STEPHENSON (1948). In order to prevent coagulation, 0.2 ml/kg of 5 per cent heparin solution was administered.

The effect of tributyrin on *transmission in the superior cervical ganglion* was studied in dial-anesthetized cats, using the technique described earlier (WRETLIND 1950).

The *acute toxicity* of tributyrin in intravenous administration was determined in mice weighing 16—23 g. Six groups of 10 animals each were used. The doses were 2, 2.5, 3, 3.5, 4 and 4.5 ml/kg of a 10 per cent emulsion of tributyrin injected intravenously. Calculation of LD_{50} was performed with the method of MILLER and TAINTER (1944).

[1] From Emulsol Corporation, Chicago, Ill.
[2] From Glidden Co., Chicago, Ill.

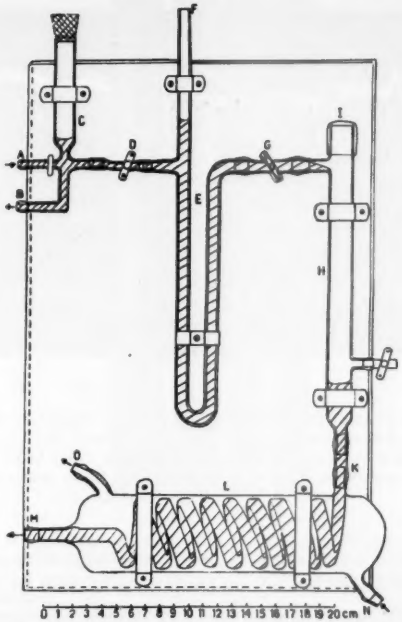

Fig. 1. *Apparatus for Starling's heart-lung preparation in the cat.* The glass apparatus is fastened to a sheet of plexiglass. The blood from the ascending aorta via the innominate artery passes through a plastic tube to the inlet A, belonging to the air-filled pressure-equilibrating vessel C, which is closed at the top by a rubber stopper. From C, a lateral tube B runs to a mercury manometer for recording the blood pressure. The blood flows from C through the rubber tube D to the U-tube E. The resistance in the rubber tube can be adjusted by a clamp, so that suitable pressure is obtained on the arterial side. Via the U-tube E, the blood passes through a rubber tube at G to the glass vessel H. The resistance to the blood flow in the rubber tube G can be regulated by a clamp. The blood level in the limb F of the U-tube can be adjusted to a suitable level by altering the resistance. If the blood flow is increased or decreased, the blood level rises or falls, this being recorded by a Marey capsule coupled to F. From the collecting vessel H, the blood flows into a glass spiral smelted into the glass tube L, through which warm water (37° C) circulates from N to O. The warmed blood then passes through the plastic tube from M into the superior vena cava of the experimental animal. If the blood volume in H becomes too large, it can be drawn off through a tube on the right. To prevent evaporation, the vessel H is covered by the glass hood I.

Results.

Fig. 2 shows the effect of rising doses of tributyrin emulsion on the *blood pressure* of the cat. When 2.5 mg (0.025 ml of 10 per cent tributyrin emulsion) were given, only a rise in blood pressure was obtained. With larger doses (5, 10 and 20 mg, respectively), a

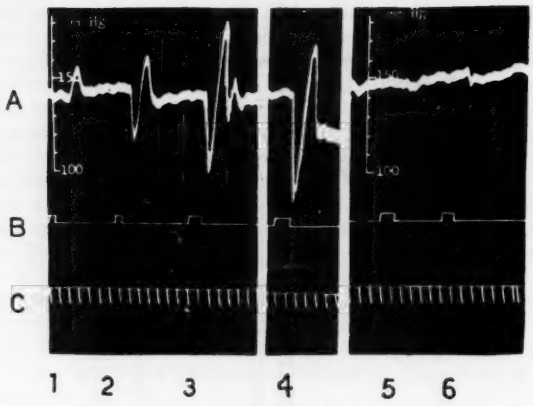

Fig. 2. *Effect on blood pressure of tributyrin emulsion and butyric acid.* Cat, 3.5 kg. At 1, 2, 3 and 4, 0.025, 0.05, 0.1 and 0.2 ml, respectively, of 10 % tributyrin emulsion was injected intravenously. At 5 and 6, injection of 0.2 and 1 ml, respectively, of an emulsion containing 1 % butyric acid. A = blood pressure. B = injection signal. C = time marking, 10 sec.

typical biphasic curve was recorded, starting with a fall in blood pressure and bradycardia, succeeded by a rise in pressure. The fact that tributyrin alone was responsible for these changes is shown by the control experiments, in which the emulsions were devoid of tributyrin but contained all the other components. Nor was the effect produced by any hydrolysis of tributyrin to butyric acid, as shown in Fig. 2, injections 5 and 6. The emulsion injected then contained no tributyrin, but 1 per cent butyric acid instead, the quantity corresponding to 10 mg of butyric acid. This had no influence whatsoever on the blood pressure.

The blood pressure effects in the cat were observed on intravenous injection of tributyrin in quantities down to 0.5 mg. The dose generally used ranged from 5 to 20 mg (0.05 to 0.2 ml of 10 per cent emulsion). The effects observed were invariably biphasic, *i. e.*, an initial fall in blood pressure, and thereafter a rise. Marked individual variations were nevertheless present. Thus, the pressor effect predominated in certain cases (Fig. 3), and in others the depressor effect (Fig. 4). Irrespective of whether the depressor effect was marked or slight, it was entirely relieved by bilateral vagotomy (Figs. 3, 4 and 11), so that only the rise in pressure persisted. These observations show that the effect on the blood pressure recorded after injection of tributyrin is a summation

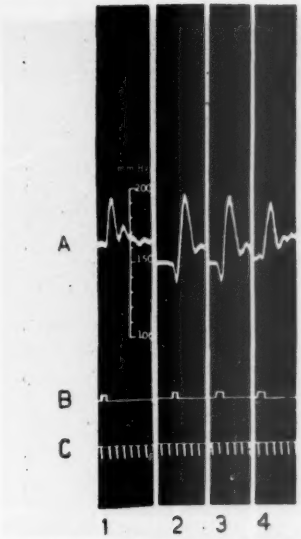

Fig. 3. *Influence of vagotomy on blood-pressure effect of tributyrin emulsion.* Cat, 4.3 kg. At 1, 2, 3 and 4, 0.1, 0.2, 0.2 and 0.2 ml, respectively, of 10 % tributyrin emulsion was injected intravenously. Between 2 and 3, right-sided vagotomy was performed, and between 3 and 4 left-sided vagotomy. A = blood pressure. B = injection signal. C = time marking, 10 sec.

curve of depressor and pressor effects. It was never possible to inhibit the fall in blood pressure by right-sided or left-sided vagotomy only.

Bradycardia and the *fall in blood pressure* disappeared after administration of atropine (Fig. 5). Another way of inhibiting the depressor effect was to inject the tributyrin directly into the aorta (Fig. 6). This was done by introducing a catheter into the aortic arch through the right carotid artery. In such experiments, only a rise in blood pressure was obtained.

Intravenous administration of procaine also prevented a fall in blood pressure and bradycardia. With a procaine dose of 10 mg/kg, administration of tributyrin was associated with a rise in blood pressure alone (Fig. 7).

The pressor effect, on the contrary, could not be inhibited. Thus, it proved resistant to tetraethylammonium, hexamethonium, dihydroergotamine, ergotamine and procaine. Moreover, it was uninfluenced by vagotomy and sinus denervation. The pressor effect persisted after evisceration and adrenalectomy. The effect

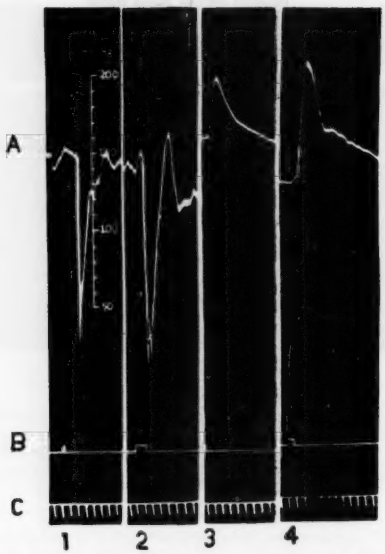

Fig. 4. *Influence of vagotomy on blood-pressure effect of tributyrin emulsion.* Cat, 3.0 kg. At 1, 2, 3 and 4, intravenous injection of 0.1, 0.2, 0.1 and 0.2 ml, respectively, of 10 % tributyrin emulsion. Between 2 and 3, bilateral vagotomy was performed. A = blood pressure. B = injection signal. C = time marking, 10 sec.

was approximately the same irrespective of the site at which injection was made. Thus, no difference was noted with injection into the veins and into the aorta. Furthermore, the same rise in blood pressure occurred irrespective of whether the injection was made through a catheter inserted in the left ventricle, in the vertebral artery, or in the carotid artery (Fig. 8). In none of these cases was a fall in blood pressure obtained, whereas this occurred, on the contrary, when tributyrin was injected intravenously.

A study of the *blood flow in the abdominal aorta* in the eviscerated, vagotomized cat showed a marked decrease in flow, together with a conspicuous rise in blood pressure, on intravenous injection of tributyrin (Fig. 9).

When tributyrin was injected into the superior vena cava in the *heart-lung preparation*, a fall in blood pressure was obtained, with an associated decrease in the blood flow from the heart (Fig. 10).

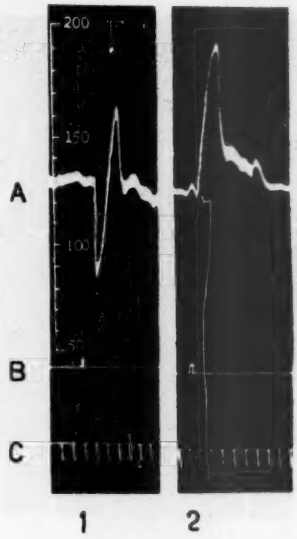

Fig. 5. *Influence of atropine on blood-pressure effect of tributyrin emulsion.* Cat, 3.5 kg. At 1 and 2, intravenous injection of 0.2 ml of 10 % tributyrin emulsion. Between 1 and 2, 0.5 mg of atropine sulfate injected intravenously. A = blood-pressure. B = injection signal. C = time marking, 10 sec.

The effect of tributyrin on respiration is shown in Fig. 8. The fall in blood pressure was associated with apnoea, followed by marked hyperpnoea. Apnoea was slight or, when injection was made into the left ventricle, vertebral artery or carotid artery, did not appear (Fig. 8). Hyperpnoea was most conspicuous with injection into the vertebral artery. Apnoea did not occur after vagotomy (Fig. 11). It was uninfluenced by atropine, procaine (Fig. 7) or dihydroergotamine.

Tributyrin had no effect on the transmission of preganglionic impulses in the *superior cervical ganglion* (Fig. 12). Thus, contraction of the nictitating membrane was the same before and during administration of tributyrin emulsion. Tetanic contraction of the nictitating membrane produced by continuous preganglionic stimulation remained unaffected by injection of tributyrin. Nor could any directly stimulating effect of tributyrin be observed.

Investigation of the *toxicity* of tributyrin on intravenous injection showed LD_{50} for mice to be 320 ± 11 mg of tributyrin/kg, or 3.2 ± 0.11 ml of 10 per cent tributyrin emulsion/kg. With

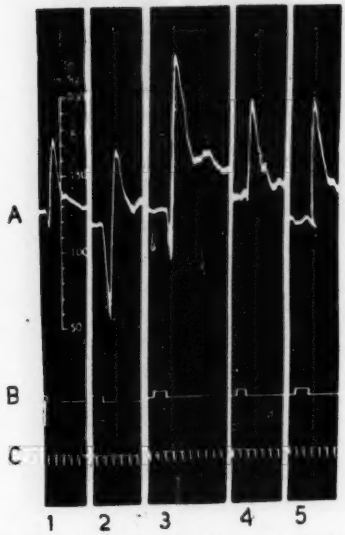

Fig. 6. *Comparison between blood-pressure effect of tributyrin emulsion injected into vein and into aorta.* Cat, 2.7 kg. At 1, 2 and 3, intravenous injection of 0.05, 0.1 and 0.2 ml, respectively of 10 % tributyrin emulsion. At 4 and 5, injection of 0.1 and 0.2 ml, respectively, of tributyrin emulsion into aorta. A = blood pressure. B = injection signal. C = time marking, 10 sec.

doses in the vicinity of LD_{50}, the characteristic symptoms were rapidly appearing convulsions and apnoea *within* 10—20 seconds of intravenous injection. The convulsions subsided after $^1/_2$—1 minute, after which the animals lay still on their side with apnoea and a slow heart rate. If respiration returned within 2—3 minutes, the animals survived.

Discussion.

As can be inferred from the results reported above, tributyrin has several pharmacological effects. As far as the changes in blood pressure are concerned, it is evident that bradycardia and the fall in blood pressure are elicited via the vagus, since they are inhibited by atropine or vagotomy. Further evidence of this fact is given by the aforementioned experiments which show that the depressor effect and bradycardia produced by tributyrin are prevented by procaine, which blocks the vagal ganglions (HAZARD 1946, ATANACKOVIC and DALGAARD-MIKKELSEN 1951). Moreover, it seems likely that these effects are evoked through receptors in the right

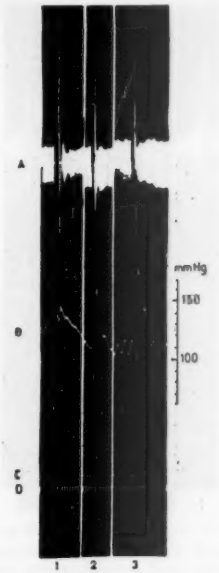

Fig. 7. *Influence of procaine on effect of tributyrin on blood pressure and respiration.* Cat, 4.0 kg. At 1, 2 and 3, intravenous injection of 0.2 ml of 10 % tributyrin emulsion. Between 1 and 2, injection of 20 mg of procaine, and between 2 and 3 injection of a further 20 mg of procaine. A = respiration. B = blood pressure. C = injection signal. D = time marking, 10 sec.

side of the heart or in the region of the pulmonary artery, since they cannot be produced by injection into the left ventricle or the central arteries. It seems as if they might be reflexes of the same nature as those described in 1867 by von BEZOLD and HIRT, and which are subsequently known as the BEZOLD-JARISCH reflex. According to DAWES (1947), bradycardia is elicited after injection of Veratrum alkaloids from receptors in the region supplied by the coronary vessels. That this cannot apply to tributyrin is shown by the experiments with injection into the left ventricle, when neither bradycardia nor a fall in blood pressure was observed. These are, on the contrary, produced in corresponding experiments with Veratridin (AVIADO, PONTIUS and SCHMIDT 1949). Evidently, it is a question of two separate receptors.

Either stimulation of the heart action or peripheral vasoconstriction can be responsible for the rise in blood pressure. It is apparent from the experiments on the heart-lung preparation that tributyrin

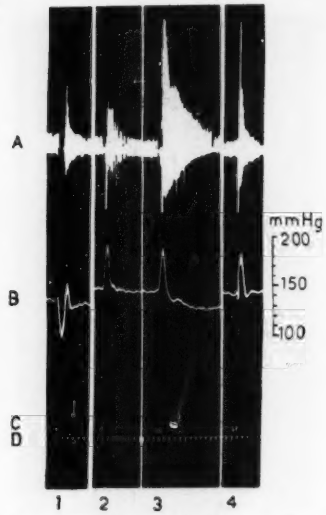

Fig. 8. *Effect of tributyrin on respiration and blood pressure.* Cat, 3 kg. At 1, 2, 3 and 4, injection of 0.05 ml of 10 % tributyrin emulsion intravenously, into left ventricle, left vertebral artery, and carotid artery, respectively. A = respiration. B = blood pressure. C = injection signal. D = time marking, 10 sec.

has no stimulating effect on the heart, but exhibits a distinct, negative inotropic effect. Moreover, it has been shown directly by determination of the flow in the abdominal aorta that the rise in pressure is associated with a decrease in blood flow. This can be explained only by pronounced peripheral vasoconstriction. This vasoconstriction does not seem to be elicited through the sympathetic system, since it is unaffected by tetraethylammonium, hexamethonium, dihydroergotamine and procaine. This supposition is strengthened by the fact that no stimulating effect can be observed on the sympathetic ganglions, such as the superior cervical ganglion.

In view of the relation between chemical structure and pharmacological effect, it is of interest to point out that tributyrin constitutes a pressor substance which contains only carbon, hydrogen and oxygen, but not nitrogen. As a rule, a pressor effect is produced by compounds containing tertiary nitrogen (amino nitrogen). Consequently, in a search for pressor substances, it is worth bearing in mind the possibility of using nitrogen-free compounds.

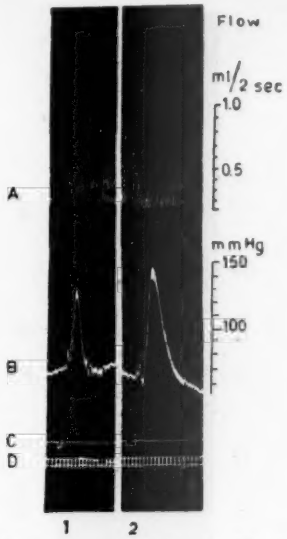

Fig. 9. *Effect of tributyrin on pressure and flow in abdominal aorta.* Cat, 3.25 kg, eviscerated and vagotomized. At 1 and 2, injection of 0.5 and 1.0 ml, respectively, of 10 % tributyrin emulsion. A = flow in abdominal aorta. B = blood pressure. C = injection signal. D = time marking, 10 sec.

The effect of tributyrin on respiration is characterized by *apnoea*, followed by hyperpnoea. Apnoea appears chiefly and most distinctly on intravenous injection, and is slight or lacking when tributyrin is injected into the left ventricle, aorta or large arteries. It seems to form part of the Bezold-Jarisch reflex, of which the triad is a fall in blood pressure, bradycardia and apnoea. The apnoea is probably elicited via receptors in the right side of the heart or in the lungs. In these cases the receptors have not yet been localized, but we can assume that it is a question of the same pulmonary chemoreceptors as those over which, according to DAWES (1947), apnoea is elicited by Veratrum alkaloids, for example. Similar apnoea is evoked by phenylguanidine and phenyldiguanide compounds (DAWES and MOTT 1950), certain isothioureates (DAWES and FASTIER 1950), 5-hydroxytryptamine (REID and RAND 1952) and other indole derivatives (ROSELL, UVNÄS and WRETLIND 1956). The hyperpnoea appearing after the apnoea may be partly reflexly elicited. Marked hyperpnoea occurs even without preceding apnoea.

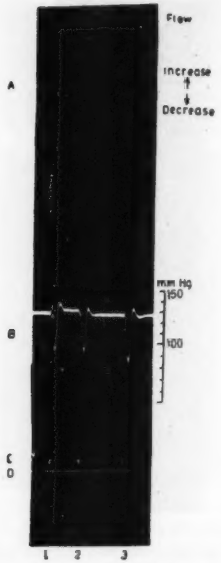

Fig. 10. *Effect on blood pressure and flow of administration of tributyrin in heart-lung preparation.* Cat, 3.9 kg, vagotomized. At 1, 2 and 3, administration of 0.4 ml of 10 % tributyrin emulsion each time. A = peripheral blood flow measured according to STEPHENSON. B = blood pressure. C = injection signal. D = time marking, 10 sec.

This indicates that it can be evoked by a direct central effect. This is supported by the fact that it is most pronounced on injection into the vertebral artery.

The relatively high toxicity of tributyrin is remarkable. Additional experiments have shown that other low-molecular neutral fats, such as tripropionin, triisovalerin and tricapronin, exhibit similar toxicity, and are by no means inert or "neutral" from the pharmacological point of view. These pharmacological investigations suggest that some of the side-effects observed in infusion of fat emulsions may be ascribed to the pharmacological effects described here. It has been shown by experiments in man that the low-molecular triglycerides in particular have severe toxic effects, such as dyspnoea, cough with expectoration of foamy sputum, and vomiting (SHAFIROFF, MULHOLLAND and BARON 1952).

It is difficult to make any statement regarding the mode of action of tributyrin emulsion on the different receptor cells.

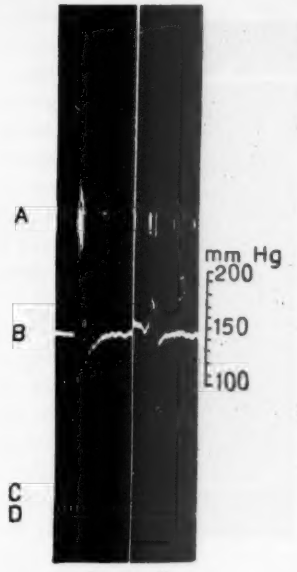

Fig. 11. *Effect of tributyrin on respiration and blood pressure.* Cat, 2.65 kg. At 1 and 2, intravenous injection of 0.1 ml of 10 % tributyrin emulsion. Between 1 and 2, bilateral vagotomy performed. A = respiration. B = blood pressure. C = injection signal. D = time marking, 10 sec.

Possibly, the small fat particles fasten in the lipophil layer of the cell surfaces, and thereby change the potential or the permeability, so that stimulation is produced. The pharmacological effect is largely dependent on the chemical properties of the fat particles, and not on the particles as such, since similar emulsions of, for example, olive oil or cottonseed oil, do not produce such effects, even if they appear alike from the physical point of view. An important matter with respect to these emulsions is whether, by changing the composition of the emulsifiers, the pharmacological effects can be altered. Since no research has yet been made in this field, no facts can be put forward with regard to this important question. Theoretically, one should be able to change the affinity of the particles to different cells or receptors by surrounding the individual fat particles by a molecular layer of various substances.

It can be recalled in this connexion that it is possible, by using

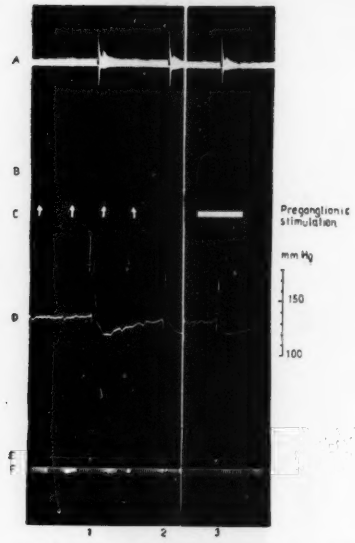

Fig. 12. *Effect of tributyrin on transmission of preganglionic impulses in superior cervical ganglion.* Cat, 2.9 kg. At 1, 2 and 3, intravenous injection of 0.1 ml of 10 % tributyrin emulsion. A = respiration. B = contraction of nictitating membrane. C = preganglionic stimulation. The single stimuli (arrows) had a duration of 10 sec. D = blood pressure. E = injection signal. F = time marking, 10 sec.

a fat emulsion that is inert from the pharmacological point of view, to charge the particles with active substances that are soluble in fat. Thus, certain alkaloids in the form of a free base, which is poorly soluble in water, can be administered intravenously in this way. This form of emulsion provides further possibilities of investigating substances that could not earlier be studied pharmacologically, owing to their poor solubility.

Summary.

The effects of tributyrin on circulation and respiration have been studied by injection of a tributyrin emulsion.

On intravenous injection, the effect of tributyrin on circulation is characterized by rapidly appearing bradycardia and a fall in blood pressure, followed by a rise in blood pressure.

Bradycardia and the fall in blood pressure are inhibited by vagotomy and procaine. They do not appear with injection into

the left ventricle, aorta or large arteries. The effect is probably elicited through receptors with afferent pathways in the vagus, and located to the right side of the heart or the lungs. The rise in blood pressure is largely due to contraction of peripheral blood vessels. This rise is not inhibited by tetraethylammonium, hexamethonium, dihydroergotamine, ergotamine or procaine.

In the heart-lung preparation, tributyrin decreases the heart action. The blood flow in the abdominal aorta is reduced, together with a rise in blood pressure after injection of tributyrin.

The effect on respiration is characterized by apnoea with a rapid onset, followed by hyperpnoea. Apnoea is not elicited after vagotomy, but appears after administration of atropine or procaine. Injection into the left ventricle, aorta or large arteries does not produce apnoea. Apnoea is probably evoked via receptors in the right side of the heart or the lungs. The hyperpnoea is considered to be produced by central stimulation.

Bradycardia, the fall in blood pressure and apnoea associated with injection of tributyrin seem to be elicited in the same way as the Bezold-Jarisch reflex described earlier.

Tributyrin is found to have neither an inhibitory nor a stimulating effect on the superior cervical ganglion.

The LD_{50} of tributyrin on intravenous injection into mice is 320 ± 11 mg/kg.

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Observations on the Action of Histamine and Related Substances on the Bronchial Resistance in the Guinea-Pig.

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Certain substances, chemically related to histamine, are known to have pharmacological actions similar to those of histamine (*e. g.* LEE and JONES 1949). The actions of such histamine-like substances — histamine analogues — are antagonized by antihistamines in the same degree as the actions of histamine (*e. g.* SCHILD 1947, LEE and JONES 1949, ARUNLAKSHANA 1953). In previous work on unanaesthetized guinea-pigs (WESTLING 1956, 1957) it was observed that two histamine-like substances, 2-β-aminoethyl pyridine and 3-β-aminoethyl-1, 2, 4-triazole, elicited respiratory symptoms closely resembling those of histamine. However, it seemed desirable to examine the effect of these substances on the bronchi in more detail.

The method devised by KONZETT and RÖSSLER (1940) was used. This method has been widely used for measuring bronchoconstrictor responses. With this method it is not possible to discriminate between changes in inflation resistance due to variations in bronchial tone and changes due to variations in pulmonary blood volume. However, it is agreed that the influence of changes in pulmonary blood volume on inflation resistance is small (BARER and NUSSER 1953, KONZETT 1956). The original method of KONZETT and RÖSSLER was slightly modified for the present experiments. The apparatus used proved very easy to handle and therefore a brief description of it will be given.

In the present experiments the bronchoconstrictor action of 2-β-aminoethyl pyridine, 3-β-aminoethyl-1, 2, 4-triazole and 1-methyl-4-β-aminoethyl imidazole was studied. In addition the influence of two histaminase inhibitors on the responses to histamine was examined.

Experimental.

Male guinea-pigs, weighing 450—900 g, were anaesthetized with a mixture of chloralose and urethane (650 mg chloralose and 8 g urethane per 100 ml), about 1.5 ml being injected per 100 g body weight. One third of the dose was given intraperitoneally and the rest subcutaneously. In some experiments pentobarbital sodium (30 mg/kg) or urethane (2 g/kg) were used.

All drugs were given intravenously through a polythene tube inserted in a jugular vein. A metal cannula was tied firmly in the trachea. The vagi were cut in the neck. In several experiments the thoracic cavity was opened by making a hole between a pair of ribs or by opening the abdominal cavity and severing the diaphragm. In some experiments the spinal cord was cut at a level between the first and second cervical vertebrae. The animal's rectal temperature was kept constant at 38° C. A contact thermometer in the rectum operated, via a relay, a lamp above the animal and an electrically heated cushion under it.

*Substances.** Histamine acid phosphate (British Drug Houses), 2-β-aminoethyl pyridine dihydrochloride, 3-β-aminoethyl-1, 2, 4-triazole dihydrochloride, 1-methyl-4-β-aminoethyl imidazole dihydrochloride, aminoguanidine sulphate (Eastman Kodak), 2-methyl-4-amino-5-methylamino-pyrimidine dihydrochloride (called B_1-pyrimidine) and mepyramine maleate (Merck & Co) were used. Doses for histamine, 2-β-aminoethyl pyridine, 3-β-aminoethyl-1, 2, 4-triazole and 1-methyl-4-β-aminoethyl imidazole are given in terms of the bases; dosages for the other drugs refer to the salts mentioned. All substances were dissolved in 0.9 % NaCl solution.

Recording of the bronchial resistance.

The "overflow" method of KONZETT and RÖSSLER (1940) was used in a slight modification. According to this method a constant volume of air is delivered by a pump, and the part of this air, which at a certain pressure does not enter the lungs, is registered by a volume recorder. An increase in the resistance of the airways towards inflation will result in larger excursions of the volume recorder. For the present experiments (fig. 1) a syringe served as a pump, the plunger being driven back and forth by a motor. Frequency and stroke volume were variable. The syringe sucked in room air and delivered it to-

* 2-β-aminoethyl pyridine, 3-β-aminoethyl-1, 2, 4-triazole and 1-methyl-4-β-aminoethyl imidazole were made available through the courtesy of dr. Reuben G. Jones, Eli Lilly Research Laboratories, Indianapolis, Indiana, U.S.A. F. Hoffman-La Roche & Co AG, Basle, Switzerland kindly supplied the B_1-pyrimidine.

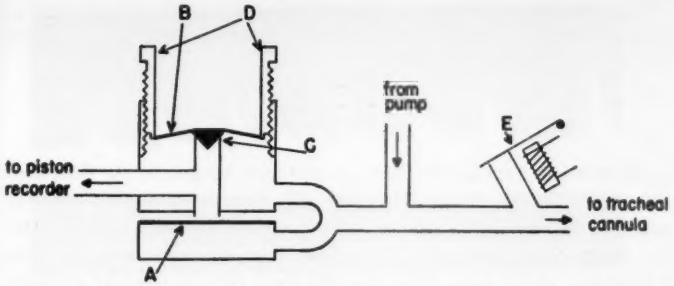

Fig. 1. Schematical drawing of apparatus for measuring bronchial resistance.

wards the animal, air flow being directed by two one-way valves. The air entered the trachea, and as the pressure rose, the flaccid rubber membrane A occluded the outlet from the piston recorder. When the pressure had reached a certain value the tense rubber membrane B yielded, thus opening the valve C so that the excess air entered the piston recorder. At "expiration" the outlet valve E, which was kept closed during "inspiration" by an electromagnet operated from contacts on the motor wheel, opened and permitted passive emptying of the animal's lungs. The rubber membrane A now permitted the emptying of the piston recorder. The tension on membrane B could be varied by screwing the metal cylinder D up or down, thereby changing the pressure necessary to open the valve C.

The ventilation pressure was varied so as to give a suitable tidal volume. The pressures necessary were measured in some experiments and found to be 8—15 cm H_2O. A ventilation frequency of 60 per min. and a tidal air of about 3 ml were used, guidance being obtained from the data of GUYTON (1947). An approximate measure of tidal air was obtained by occluding the tube leading to the animal immediately before an "inspiration", the increase in excursion of the piston recorder during the following "inspiration" being noted. In the figures in the present paper volume calibrations for the tidal air are given, "zero level" denoting the excursion of the lever after occlusion of the tube leading to the animal.

The piston recorder had an internal diameter of 31 mm. It was connected to a lever, the point of which was writing through an arch with 29 cm radius. The magnification was about 13: 1. In the calibrations and in measuring the responses the curvilinearity of the excursions were ignored. For quantitative comparisons the height of the responses was measured, *i. e.* the vertical distance between the highest excursion and the basal level immediately before the injection.

One difficulty often encountered was a slow rise of the base line, *i. e.* a diminishing tidal air. This was mostly noticed in the beginning of an experiment. If the ventilation pressure was then slightly increased a stable base-line could often be maintained. However, small changes in the basal level did not appear to influence the responses to histamine.

The responses were essentially the same in spinal animals and in animals with opened thorax. However, these procedures shortened the survival considerably.

Results.

Histamine regularly gave a distinct response in doses of about 0.5 μg per animal (fig. 2). The dose-response curve was often steep and the height of the responses seemed to be linearly related to the dose of histamine, rather than to the logarithm of the dose. This was the case irrespective of whether the volume or the concentration of the solution was varied.

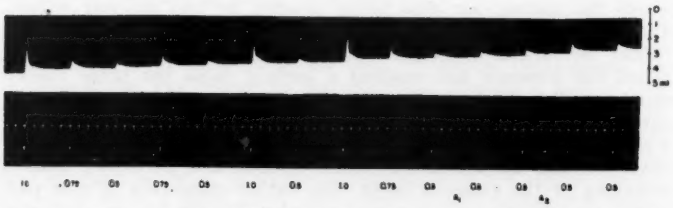

Fig. 2. Effect of different doses of histamine on bronchial resistance. The figures denote amounts of histamine (µg) injected intravenously. Aminoguanidine was given intravenously at A_1 (1 mg/kg) and at A_2 (10 mg/kg). Guinea-pig 500 g. Chloralose-urethane. Time in min. Tidal air in ml.

It was found that the three histamine-like substances tested had an action like histamine upon the bronchial resistance. The dose-response curves for the histamine-like substances were similar to that of histamine. The ratio of equiactive doses of 2-β-aminoethyl pyridine and histamine varied between 30:1 and 44:1. 3-β-aminoethyl-1, 2, 4-triazole was more active than the pyridine compound, the ratio between equiactive doses of the triazole compound and histamine being 7—10:1. Fig. 3 shows a comparative assay of 3-β-aminoethyl-1, 2, 4-triazole and histamine, two doses of each drug being tested. In this assay the triazole compound was 8.9 times less active than histamine. The effect of 1-methyl-4-β-aminoethyl imidazole was examined in two experiments. The methylated derivative was about 200 times less active than histamine (fig. 4).

Mepyramine given by vein antagonized the action of histamine, 2-β-aminoethyl pyridine and 3-β-aminoethyl-1, 2, 4-triazole equally well. Doses of 0.5—1.0 μg of mepyramine reduced the sensitivity to these substances to 1/2 or 1/3 of that before

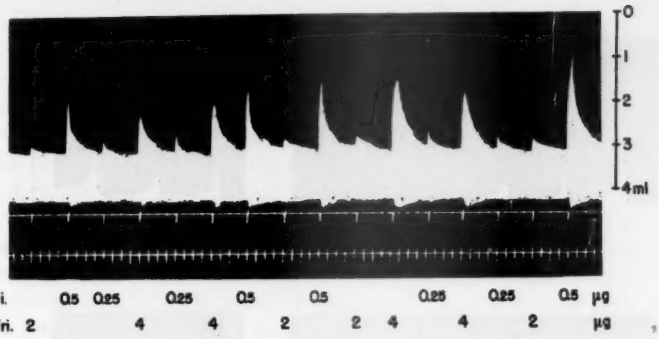

Fig. 3. Comparison of the action of histamine (Hi) and 3-β-aminoethyl-1, 2, 4-triazole (Tri). 2 × 2 assay. Guinea-pig 500 g. Chloralose-urethane. Time in min. Tidal air in ml.

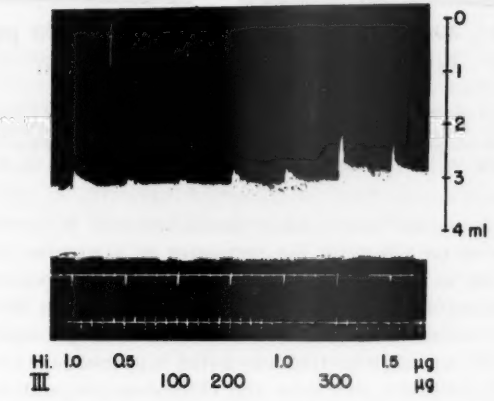

Fig. 4. Comparison of the action of histamine (Hi) and 1-methyl-4-β-aminoethyl imidazole (III). Guinea-pig 500 g. Chloralose-urethane. Time in min. Tidal air in ml.

mepyramine. Fig. 5 shows the effect of a larger dose, 20 μg, of mepyramine. This dose reduced the sensitivity to histamine and to the analogues to the extent that about 33 times more of each drug had to be given in order to obtain the same response as before mepyramine. The effect of mepyramine on the response to 1-methyl-4-β-aminoethyl imidazole was not examined.

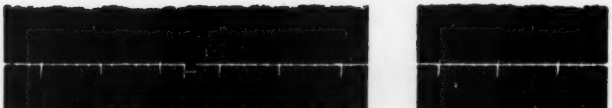

Fig. 5. Effect of mepyramine on responses to histamine (Hi), 2-β-aminoethyl pyridine (Py) and 3-β-aminoethyl-1, 2, 4-triazole (Tri). 20 µg of mepyramine was injected intravenously at M. Interval between tracings about 20 min. Guinea-pig 500 g. Chloralose-urethane. Time in min. Tidal air in ml.

Histaminase inhibitors, aminoguanidine and B_1-pyrimidine, had little or no effect on the responses to histamine in these experiments in spite of the fact that the dose-response curve for histamine was steep (fig. 2). Doses from 0.1 to 50 mg/kg of the inhibitors were used. The absence of conspicuous potentiation with aminoguanidine was noted in guinea-pigs anaesthetized with urethane, urethane and chloralose, or pentobarbital sodium.

Discussion.

The observation that 2-β-aminoethyl pyridine and 3-β-aminoethyl-1, 2, 4-triazole acted like histamine on the bronchial resistance in the anaesthetized guinea-pig adds to previous evidence that these substances have pharmacological actions very similar to those of histamine. The parallel decrease in responsiveness to histamine and the two histamine analogues

after mepyramine is also in agreement with earlier observations on isolated tissues (*e. g.* LEE and JONES 1949, ARUNLAKSHANA 1953) and intact animals (LINDELL and WESTLING 1956, WESTLING 1957).

The action of 1-methyl-4-β-aminoethyl imidazole was examined because it has recently been shown (SCHAYER and KARJALA, 1956) that methylation of histamine, leading to the formation of the compound mentioned, is in many species an important step in the metabolism of histamine. LEE and JONES (1949) found that the activity of 1-methyl-4-β-aminoethyl imidazole on the cat's blood pressure and on the guinea-pig's isolated ileum was only 0.2 % and 0.6 %, respectively, of that of histamine. In the present experiments the methylated derivative was about 200 times less active than histamine. Clearly, the methylation of histamine shown by SCHAYER and his coworkers to occur in many species, must be an important means of inactivation of histamine.

In the present investigation two histaminase inhibitors, aminoguanidine and B_1-pyrimidine, did not modify histamine effects distinctly although they were given in a dosage, effective in potentiating histamine effects in other types of experiments (LINDELL and WESTLING 1956, WESTLING 1956, 1957). In a previous report (LINDELL and WESTLING 1954) it was noted that a different histaminase inhibitor, stilbamidine, potentiated the effect on the bronchial resistance of histamine given by vein to guinea-pigs. Further work is needed to clarify whether the discrepancy is due to different experimental conditions or to differences between stilbamidine and the histaminase inhibitors used in the present work.

Summary.

1. The action of 2-β-aminoethyl pyridine, 3-β-aminoethyl-1, 2, 4-triazole and 1-methyl-4-β-aminoethyl imidazole on the bronchial resistance was studied in anaesthetized guinea-pigs.

2. The substances examined acted like histamine and their actions were antagonized by mepyramine in the same degree as was that of histamine.

3. Two histaminase inhibitors, aminoguanidine and B_1-pyrimidine, did not potentiate the actions of histamine.

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It is a pleasure to acknowledge the contribution of Mr. B. CLEMENTZ, Chief engineer at the institute's workshop, who designed and built the pressure regulator. Skilful technical assistance was given by Miss M.-B. JOHANSSON.

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Aus dem Physiologischen Institut der Universität
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Eine oximetrische Methode zur Kreislaufzeitmessung am menschlichen Gehirn.

Von

NILS JOHAN NILSSON.

Eingegangen am 14. März 1957.

Seit 1826, als HERING die ersten Messungen von Kreislaufzeiten ausführte, ist bei der Mehrzahl der Untersuchungen das von ihm eingeführte Messprinzip benutzt worden. Durch schnell aufeinanderfolgende Blutentnahmen an einer Stelle der Blutbahn wird der Zeitpunkt der Ankunft einer leicht identifizierbaren Substanz festgestellt, die an einem anderen Punkt in den Kreislauf injiziert wird. Die älteren Untersucher, wie HERING (1829, 1832, 1853), VOLKMANN (1850) und VIERORDT (1862), haben hauptsächlich die Messung der Zeit eines vollständigen Kreislaufs angestrebt, in der Annahme, dass die Kreislaufzeiten aller einzelnen Blutportionen nahezu gleich seien. Diese Auffassung hat sich allmählich als unrichtig erwiesen, und die Messung der totalen mittleren Kreislaufzeit hat damit an Interesse verloren. Die vor allem durch STEWART (1897, 1921 a) und HAMILTON, MOORE, KINSMAN und SPURLING (1928) weiterentwickelte Farbstoffverdünnungsmethode ist dabei hauptsächlich zur Bestimmung von Blutdurchströmungen — vor allem des Herzminutenvolumens — gebraucht worden. Für die Messung von Kreislaufzeiten, besonders der zentralen Teile des Kreislaufs, sind mehrere Methoden vorgeschlagen worden, von denen einige in der Klinik Verwendung gefunden haben (Übersicht s. JÖNSSON 1951).

Die photoelektrische Methode der Messung der Lichtdurchlässigkeit des Blutes, die Oximetrie, ist bei Untersuchungen dieser Art durch ihre Objektivität und die Möglichkeit einer kontinuierlichen Registrierung in vielen Fällen sehr geeignet. Für die Messung von Kreislaufzeiten sind mehrere Varianten der oximetrischen Methode angegeben:

1) Gleichzeitige Registrierung der Lichtdurchlässigkeit an zwei hintereinander in der Strombahn gelegenen Stellen und Abmessung der Zeit zwischen korrespondierenden Punkten der Kurven (KRAMER 1935, 1936).

2) Registrierung der Sauerstoffsättigung an einer Stelle und Messung der Zeit von dem Anfang eines schnellen Atemzuges, der die Sättigung des Arterienblutes ändert, bis zum Auftreten dieser Änderung in der Oximeteranzeige (MATTHES und MALIKIOSIS 1936).

3) Bei der gleichen Anordnung wie unter 2) Registrierung der Extinktion eines in die Blutbahn injizierten Farbstoffes bei möglichst konstanter Sauerstoffsättigung (MATTHES und SCHLEICHER 1939).

Die erstgenannte Methode mit doppelter kontinuierlicher Registrierung, die bei Tierversuchen sehr praktisch sein kann (s. z.B. KRAMER, HOTOP, NILSSON, GEHL und ULLRICH 1952), hat beim Menschen bisher keine Verwendung gefunden.

Die zweite Ausführungsform ist von MATTHES und seinen Mittarbeitern in umfangreichen Arbeiten gebraucht worden (Übersicht s. MATTHES 1937, 1951). In etwas vereinfachter Form wurde sie von WEXLER und WHITTENBERGER 1946 wieder aufgegriffen und später in verschiedenen Variationen von mehreren Forschern angewendet (z.B. FOWLER und COMROE 1948, ELAM, ROOS und NEVILLE 1948, LINDGREN 1948, WOOD, KNUTSON und TAYLOR 1949, WOOD, TAYLOR und KNUTSON 1949, KNUTSON, TAYLOR und WOOD 1949, KNUTSON, TAYLOR, ELLIS und WOOD 1950, CALLEBAUT, LEQUIME and DENOLIN 1950). WILLIAMS beschreibt sie 1956. — Die Methode hat sich als sehr brauchbar erwiesen. Allerdings muss die Lunge immer Ausgangspunkt der Messung sein.

Die dritte Variante der Methodik ist in dieser Hinsicht allgemeiner verwendbar, doch ist die dabei benötigte Injektion manchmal unbequem und in gewissen Fällen für die Messung störend. Die Methode ist ausser von MATTHES (s. oben) von vielen Forschern gebraucht worden, für die Bestimmung der Zeit Cubitalvene — Ohr (JABLONS 1943, JABLONS und COHEN

1943, JABLONS, COHEN und SWIRSKY 1944, MILLER 1948, WOOD, KNUTSON und TAYLOR 1949, KNUTSON und Mitarb. 1950), für die Bestimmung des Herzminutenvolumens (NICHOLSON und WOOD 1950, BEARD, NICHOLSON und WOOD 1950, NICHOLSON, BURCHELL und WOOD 1951, NICHOLSON und WOOD 1951, BEARD und WOOD 1951, WARNER und WOOD 1952) und für vergleichende Zeitbestimmungen an Ohr, A. radialis und A. femoralis (BEARD, WOOD und CLAGETT 1951). Auch FRIEDLICH, HEIMBECKER und BING 1950, MILNOR, TALBOT, MCKEEVER, MARYE und NEWMAN 1953 und SHADLE, FERGUSON, GREGG und GILFORD 1953 benutzen das gleiche Prinzip. Ebenso sind einzelne Untersuchungen im arteriellen System mit dieser Methode ausgeführt worden (CORBOZ 1948). LOCHNER und SCHOEDEL (1950) und HELLER, LOCHNER und SCHOEDEL (1951) injizieren ausser Farbstoff in ähnlicher Weise auch farblose Flüssigkeit (Plasma, Kochsalzlösung), um die Verdünnung des Hämoglobins zu registrieren.

Die Kreislaufzeiten peripherer Organe haben im Vergleich wenig Interesse erregt. Die ausführlichsten Studien stammen von STEWART (1921 c) und umfassen Milz, Niere, Darm, Herz und Retina. *Das Gehirn* ist in dieser Hinsicht nur in sehr beschränktem Umfang untersucht worden. WOLFF und BLUMGART (1929) haben durch Injektion radioaktiver Stoffe die Zeit von der A. carotis bis zum Herzen bestimmt. Mit Röntgenaufnahmen und -durchleuchtung bei Carotis-Angiographien sind Zeitstudien seit MONIZ (1940) von vielen Forschern ausgeführt worden (s. Übersicht bei GREITZ 1956). GREITZ (1956) hat ausführliche Untersuchungen mit beiden oben genannten Methoden durchgeführt. LASSEN und MUNCK (1955) ziehen aus den Konzentrationskurven des Kryptongases bei Gehirndurchblutungsbestimmungen *ad modum* KETY (KETY und SCHMIDT 1945, 1948 a) Schlüsse auf die cerebrale Kreislaufzeit.

Im folgenden wird ein Verfahren zur Messung cerebraler Kreislaufzeiten beim Menschen beschrieben, das auf oximetrischer Methodik aufgebaut ist.

Methode.

Als Prinzip dient die oben beschriebene gleichzeitige Registrierung der Sauerstoffsättigung des Blutes an zwei Punkten der Strombahn, an der Ein- bzw. Austrittsstelle am

Gehirn. Als arterieller Messpunkt wird das Ohr benutzt; die Messung geschieht mit einem Ohroximeter. Die Sättigung des Venenblutes wird mit einer Cuvetteneinheit gemessen, die direkt auf einer Kanüle sitzt, mit der — wie bei der Durchblutungsuntersuchung nach KETY und SCHMIDT — der Bulbus jugularis punktiert wird.

Das Oximeter ist in einer früheren Arbeit (NILSSON 1956) beschrieben. Die *Cuvetteneinheit* enthält 4 Photoelemente, abwechselnd rot- und infrarot-empfindlich. Sie ist nur 2 cm lang und dadurch, sowie durch die Anbringung unmittelbar an der Punktionskanüle, imstande, die Sättigungsveränderungen im Jugularisblut ohne nennenswerte zeitliche Verspätung anzugeben.

Um ein gleichmässiges Absaugen des Blutes aus der Vene zu erreichen, brauchen wir, wie BERNSMEIER und SIEMONS (1953), spezialkonstruierte Rekordspritzen (à 50 ml), deren Stempel mit Gewinde versehen sind und mit Kegelrädern von elektrischen Synchronmotoren mit konstanter Geschwindigkeit herausgezogen werden. Diese Anordnung, bei der das Stromvolumen direkt konstant gehalten wird und unmittelbar kontrolliert werden kann, ist der häufig verwendeten Methode des Absaugens mit konstantem Vakuum bedeutend überlegen. Um die Absaugung kontinuierlich durchführen zu können, brauchen wir zwei solcher Spritzen mit Motoren, die durch starre Polyaethylenschläuche* über einen Dreiwegehahn alternierend mit der Cuvetteneinheit verbunden werden können.

Das Blut fliesst unmittelbar nach der Passage durch die Einheit durch ein kurzes Glasrohr, das weiter als die Schläuche ist und Natriumoxalatkristalle enthält. Das Oxalat wird erst nach der Cuvetteneinheit angebracht, um die Einheit möglichst nahe an die Vene zu plazieren und um eventuelle optische Einwirkung der sich in das Blut auflösenden Kristalle zu vermeiden. Als Sauggeschwindigkeit hat sich 5 ml pro Minute als ausreichend erwiesen. Durch diese niedrige Geschwindigkeit wird der Blutverlust des Untersuchten unbedeutend und die Gefahr einer Aspiration von nicht-cerebralem Blut aus dem zentralen Teil der V. jugularis verringert. Die Konstanz der Sauggeschwindigkeit eliminiert den Einfluss des bei der gebrauchten Kompensationsschaltung ohnehin geringen Strömungseffektes auf die Anzeige des Cuvettenoximeters.

* Wir danken der Firma Ferrosan, Malmö-Kopenhagen, für freundliches Überlassen von Polyaethylenmaterial in verschiedener Ausführung.

Die Verspätung der Anzeige des Cuvettenoximeters ist in Modellversuchen untersucht worden, in denen Blut mit der beschriebenen Anordnung durch Punktion aus einem grosskalibrigen, dünnwandigen Polyaethylenschlauch gesogen wurde, bei gleichzeitiger Messung der Sättigung im Schlauch an der Punktionsstelle mit Hilfe einer Ohreinheit. Die Zeitdifferenz der Angaben der beiden Einheiten betrug 0.4—0.7 durchschnittlich 0.5 sec.

Die lineare Anzeige des Oximeters ermöglicht es, vor der Untersuchung auf verhältnismässig einfache Weise eine Eichung vorzunehmen, indem man erst vollgesättigtes, dann mit Natriumdithionit reduziertes Blut der zu untersuchenden Person durch die Einheit saugt und dabei durch Regelung der Kompensation und der Verstärkung den Galvanometerausschlag auf 100 bzw. 0 einstellt.

Über die quantitative Übereinstimmung der mit einem *Ohroximeter* registrierten Kurven mit gleichzeitig aufgenommenen Kurven einer Cuvetteneinheit ist schon mehrfach berichtet worden (KNUTSON, TAYLOR und WOOD 1949, WOOD, TAYLOR und KNUTSON 1949, BEARD, NICHOLSON und WOOD 1950, NICHOLSON und WOOD 1951, BEARD und WOOD 1951, MILNOR und Mitarb. 1953).

Auch zeitliche Zusammenhänge sind studiert worden (WOOD, TAYLOR und KNUTSON 1949, NICHOLSON, BURCHELL und WOOD 1951, BEARD, WOOD und CLAGETT 1951), bisher doch nur an Arterien, die verhältnismässig fern vom Ohr liegen, wie die A. radialis oder femoralis.

Für die vorliegende Aufgabe wurde deshalb eine direkte Vergleichsuntersuchung über den Verlauf der Sättigungskurven im Ohr und in der A. carotis communis durchgeführt, indem diese punktiert und die oben für die Venenblutmessung beschriebene Anordnung mit Cuvetteneinheit und Motorspritze an die Punktionskanüle angeschlossen wurde.

Wie in der Abb. 1 dargestellt, sind die Sättigungskurven sowohl zeitlich wie in ihrem allgemeinen Verlauf beinahe identisch. Die Zeitdifferenz zwischen korrespondierenden Punkten der Carotis- und Ohrkurve betrug bei 25 Messungen an 9 Personen 0.0—1.1, im Durchschnitt 0.2 sec. Die Angaben der Ohreinheit sind also im Verhältnis zur A. carotis um praktisch den gleichen Betrag verspätet wie die Angaben der Cuvetteneinheit im Verhältnis zur V. jugularis. Eine besondere Korrektion ist somit nicht nötig, sondern die abgelesenen

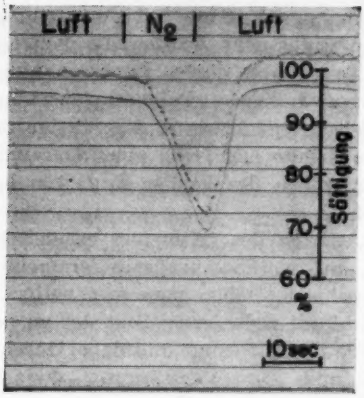
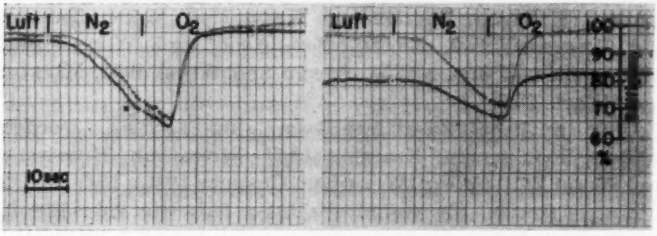


Abb. 1. Zeitliche Beziehungen zwischen A. carotis communis und Ohr. Obere Kurve: O_2-Sättigung des Carotisblutes; Cuvettenoximeter. Untere Kurve: Ohroximeter, approximative Eichung rechts.



a b

Abb. 2. Gleichzeitige Registrierung der arteriellen Sättigung an beiden Ohren der Versuchsperson. Obere Kurve: Rechtes Ohr, Ohreinheit auf gewöhnliche Weise angebracht, Eichung rechts. Untere Kurve: Linkes Ohr; keine Eichung. In a) ist die linke Ohreinheit ganz lose, in b) sehr fest an das Ohr angedrückt.

Zeitdifferenzen Ohreinheit — Cuvetteneinheit können den Differenzen Carotis — Jugularis gleichgesetzt werden.

Eine in der Ohroximetrie bisher nicht beachtete Frage ist die des eventuellen mechanischen Einflusses der Einheit auf die Durchströmung des Ohres. Die Ohreinheit muss zum Zwecke der Fixation das Ohr mit einem gewissen, wenn auch leichten Druck zwischen den Fenstern im Lichtweg fassen, und da dieser Druck naturgemäss nicht von Fall zu Fall völlig konstant gehalten werden kann, könnte die Möglichkeit bestehen, dass ein festeres Andrücken der Einheit zu einer

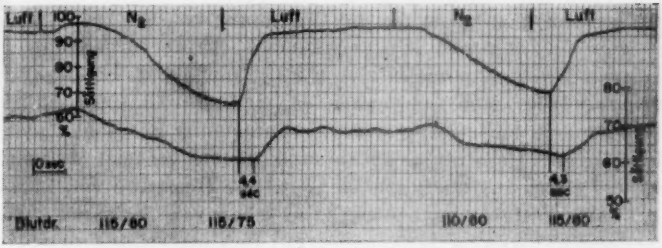


Abb. 3. Beispiel einer registrierten Kurve mit zwei Kreislaufzeitmessungen. Obere Kurve: Ohreinheit, Eichung links. Untere Kurve: Cuvetteneinheit, an den Bulbus jugularis angeschlossen, Eichung rechts. Unten auskultatorisch gemessener Blutdruck. Die Versuchsperson atmet abwechselnd Luft und Stickstoff nach den Angaben am oberen Rande des Bildes.

Verlangsamung der Strömung führe. Ein Versuch zur Klärung dieser Frage ist in der Abb. 2 wiedergegeben. Hier ist die Sättigung gleichzeitig an beiden Ohren der Versuchsperson registriert worden, wobei die eine Ohreinheit auf normale Weise angebracht und unverändert gelassen wurde, während die andere abwechselnd fester und loser angedrückt wurde. Wie aus der Abbildung ersichtlich, wird die Grösse des Ausschlages (bei unveränderter Verstärkung) durch starkes Andrücken erheblich vermindert, der zeitliche Verlauf der Kurve aber nicht beeinflusst.

Ein Beispiel einer Messkurve ist in der Abb. 3 abgebildet. Die durch den Gebrauch von elektronischer Verstärkung ermöglichte Anwendung schnellschwingender Spiegelgalvanometer (Pencil-Galvanometer der Firma Gebr. Ruhstrat, Göttingen, innerer Widerstand 7 Ω, Eigenfrequenz 5 Hz) garantieren eine zeitgetreue Registrierung der Sättigungsänderungen in den Messeinheiten.

Genauigkeit der Methode.

Wenn der zeitliche Abstand zwischen zwei einander entsprechenden Punkten der registrierten Sättigungskurven gemessen werden soll, liegt es auf der Hand, dass die Ablesegenauigkeit um so grösser wird, je plötzlicher der Kurvenausschlag auftritt. Da eine Erhöhung des Sauerstoffpartialdruckes in der Alveolarluft schneller durchgeführt werden kann als eine Senkung, eignet sich die plötzliche Einatmung von Sauerstoff

zu diesem Zweck besser als die Einatmung von Stickstoff, wie sie von WEXLER und WHITTENBERGER (1946) vorgeschlagen und von KNUTSON und Mitarb. (1950), sowie CALLEBAUT und Mitarb. (1950), zum Teil verwendet wurde. Dieser Unterschied ist besonders nach längerer Sauerstoffatmung zu beobachten; dabei kann Stickstoff mehr als eine Minute geatmet werden, ehe die Sättigung zu fallen anfängt. Der Übergang von Luft- auf Sauerstoffatmung bewirkt, wie schon von MATTHES 1935 dargestellt, einen im allgemeinen gut definierten Anfangszeitpunkt der Sättigungserhöhung in der Arterienkurve. In der Venenkurve ist der entsprechende Zeitpunkt dagegen im allgemeinen nicht definierbar.

Wegen dieser, durch die Passage durch das Gehirn verursachten „Glättung" der venösen Sättigungskurve im Verhältnis zur arteriellen, ist es notwendig, verhältnismässig grosse Sättigungsveränderungen vorzunehmen, um leicht ablesbare Ausschläge in der Venenkurve zu erhalten. Wir haben deshalb im allgemeinen die Versuchsperson kurze Zeit Stickstoff atmen lassen, bis die Sättigung des Arterienblutes auf etwa 70 % herabgesunken war, um dann Luft oder Sauerstoff zu geben. Die dabei entstehenden Knickpunkte sind in der Ohrkurve sehr scharf, in der Venenkurve etwas abgerundet, aber in den meinsten Fällen gut definiert. Da bei gewissen Personen spontane Schwankungen in der Venensättigung vorkommen, die den Verlauf der Kurve am Umkehrpunkt entstellen können, empfiehlt es sich, wiederholte Messungen vorzunehmen. Die Zeiten werden auf 0.1 sec abgelesen, was im allgemeinen ohne Schwierigkeit möglich ist. Die durchschnittliche Differenz zwischen aufeinanderfolgenden Messungen betrug im ganzen Material 0.4 sec; diese Zahl umfasst sowohl Methodenfehler wie etwaige spontane Schwankungen der Durchströmungsgeschwindigkeit.

Eine Frage, die bei Bestimmungen mit dieser Methode berücksichtigt werden muss, ist die, ob der Messvorgang die Kreislaufzeit beeinflusst, da ja die Stickstoffgaben eine vorübergehende cerebrale Anoxie verursachen werden. Es ist bekannt (KETY und SCHMIDT 1948 b), dass Einatmung von sauerstoffarmen Gasgemischen die Gehirndurchblutung steigert. Über die für das Auftreten dieser Wirkung nötige Latenzzeit ist aber wenig bekannt, da die Stickoxydulmethode darüber keine Auskunft geben kann. Nach den tierexperimentellen Untersuchungen von NOELL und SCHNEIDER (1942) „beträgt

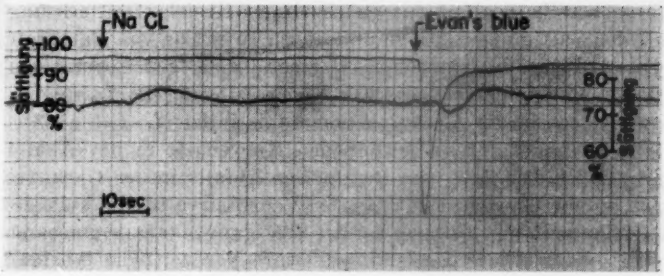


Abb. 4. Durchlässigkeit des Ohr- und Jugularisblutes bei intraarteriellen Injektionen. Bei den Pfeilen werden 2 ml physiologische Kochsalzlösung bzw. 2 ml Lösung von Evans blue in die A. carotis communis injiziert. Man beachte die 6—30 sec nach der NaCl-Injektion auftretende Sättigungszunahme in der Venenkurve, die sich nach der Farbstoffinjektion wiederholt.

die Latenz bei einem arteriellen O_2-Gehalt von 13 bis 17 Vol.-% über 1 Minute". Da bei den von uns gebrauchten Stickstoffgaben gewöhnlich arterielle Mindestsättigungen von 70 bis 60 % in 25—40 sec erreicht werden, sind störende Einflüsse kaum zu erwarten. In einigen Versuchen haben wir, um eine Auffassung von diesen Verhältnissen zu bekommen, an die einleitende Stickstoffatmung eine O_2-Mangelatmung für kürzere oder längere Zeit angeschlossen. Es hat sich dabei gezeigt, dass nach Einatmung während 2—5 min von Mischungen mit 11.4—6.0 % O_2 die Kreislaufzeit beim Übergang zur Luftatmung merkbar verkürzt wird. Bei O_2-Mangelatmung von kürzerer Dauer als 2 min war die Veränderung inkonstant, bei einer Dauer unter 1 min wurde sie nicht beobachtet.

Auch eine Beeinflussung des Blutdruckes durch den Messvorgang kommt als Fehlerquelle in Betracht. Bisweilen können kleine Veränderungen beobachtet werden (vgl. Abb. 3). Ein systematischer Einfluss liegt aber nicht vor: Bei 39 Kreislaufzeitmessungen an 22 Personen war der auskultatorische Blutdruckwert im Durchschnitt vor dem Stickstoffatmen 134/87 und während desselben — bei der Kreislaufzeitmessung — 135/86 mm Hg.

Farbstoffmessungen.

Um die Abhängigkeit der gemessenen „kürzesten" Kreislaufzeit von der mittleren näher zu untersuchen, wurde eine

Versuchsreihe geplant mit gleichzeitiger Kreislaufzeitmessung mit der Sättigungsmethode und durch Injektion von Farbstoff (Evans blue). Es wurden 2 ml einer 0.2 %-igen Lösung von Evans blue in physiologischer Kochsalzlösung im Laufe von etwa ½ sec in die A. carotis communis injiziert.

Ein Beispiel der so gewonnenen Kurven ist in der Abb. 4 dargestellt. Die Zeit vom Beginn des Abfalls in der Ohrkurve zum entsprechenden Punkt in der Venenkurve ist die kürzeste Kreislaufzeit, wie sie auch bei der Sättigungsmethode gemessen wird; die mittlere Kreislaufzeit wäre der zeitliche Abstand zwischen den Schwerpunkten der von den Kurvenausschlägen beschriebenen Flächen (vgl. Hamilton und Mitarb. 1932, Meier und Zierler 1954). Bei der photometrischen Analyse von Evans blue im strömenden Blut muss beachtet werden, dass ein Ausschlag des Oximeters sowohl durch Änderungen der Farbstoffkonzentration wie der Sauerstoffsättigung des Hämoglobins hervorgerufen werden kann, eine Tatsache, die das Oximeter für die Messung von Farbstoffkonzentrationen in den Venen, wo Sättigungsänderungen nicht ausgeschlossen werden können, weniger geeignet macht. Vielfach ist jedoch die Sättigung des Venenblutes, wie in dem hier abgebildeten Fall, so konstant, dass trotz dieser Einschränkung eine wenigstens annähernd richtige Messung der Konzentration des Farbstoffes erwartet werden könnte. Wie die Abb. 4 zeigt, wird aber der vom Farbstoff hervorgerufene, nach unten gerichtete Ausschlag in der Venenregistrierung sofort von einer nach oben gerichteten Welle gefolgt, so dass eine diphasische Reaktion entsteht. Nach der vorhergehenden Injektion von 2 ml Kochsalzlösung entsteht ein ganz analoger Ausschlag nach oben, der sich über die Zeit 6—30 sec nach der Injektion ausstreckt und eine maximale Amplitude entsprechend ungefähr 5 % Sättigung hat. Diese Reaktion durch eine Verdünnung des Blutes zu erklären erscheint nicht möglich wegen der kompensierenden Eigenschaften des Oximeters und der Grösse des Ausschlages im Verhältnis zu dem kleinen injizierten Volumen, vor allem aber durch die Abwesenheit der Reaktion in der arteriellen Kurve und ihre Persistenz bei der Injektion der stark absorbierenden Farbstofflösung. Die Erscheinung bezeichnet offenbar eine durch die Injektion verursachte vorübergehende Zunahme der Venensättigung, die von einer entsprechenden Erhöhung der Durchblutung hervorgerufen sein dürfte.

Diese Reaktion trat bei 7 von 9 untersuchten Personen auf. Auf eine weitere Fortsetzung der Untersuchungsserie wurde verzichtet, weil damit gerechnet werden musste, dass eine nicht unwesentliche Erhöhung der Gehirndurchblutung gerade im Augenblick der Messung stattfände. Auch wenn man eine „Korrektion" des diphasischen Kurventeils durch Transponierung des Sättigungsausschlages von der Kochsalzinjektion versuchen würde — was nur annähernd möglich ist, weil die Veränderungen nicht jedesmal identisch sind — hätte man dadurch eine Aussage über den Zustand während der Mehrdurchblutung bekommen.

Untersuchungen zur näheren Erklärung dieses Phänomens sind nicht vorgenommen worden. Es kann jedoch auf die Feststellung FOLKOWS (1953) hingewiesen werden, dass intraarterielle Injektionen von hämolysiertem Blut, auch in Quantitäten von nur einzelnen Kubikmillimetern, beträchtliche Gefässdilatationen am isolierten Katzenhinterbein hervorrufen, und dass deutliche Durchblutungssteigerungen überhaupt nach intraarteriellen Injektionen entstehen, wenn diese nicht sehr langsam und vorsichtig gegeben werden. FOLKOW vermutet als Ursache eine minimale Hämolyse, die zur Freisetzung gefässdilatierender Stoffe (ATP ?) aus den Erythrozyten führt.

Da bei der Farbstoffverdünnungsmethode schnelle Injektionen essentiell sind, führt die beschriebene Beobachtung zu dem Schluss, dass Injektionsmethoden mit Injektionen in die A. carotis für die Untersuchung des Gehirnkreislaufs ungeeignet sind. Bei der von NYLIN und BLÖMER (1955) ausgeführten Untersuchung dieser Art wurden dementsprechend auffallend hohe Werte für die Gehirndurchblutung gefunden.

Diskussion.

Zu den Vorteilen der hier beschriebenen Methode gehört der Umstand, dass keine Injektion von Indikatorstoff notwendig ist, die nicht nur die Messprozedur kompliziert, sondern u.U. das Resultat beeinflussen kann (vgl. oben). Einen besonderen Vorzug hat die Methode darin, dass die Messungen oft wiederholt und beliebig viele Male durchgeführt werden können. Auch wenn mehrere Bestimmungen mit Hilfe kurzdauernder Stickstoffatmung in Abständen von etwa 1 min ausgeführt werden, wird keine systematische Veränderung der Zeiten beobachtet.

Bei spontan langsamer oder unregelmässiger Atmung und Hypoxämie können manchmal Zeitablesungen ohne besondere Eingriffe häufiger ausgeführt werden. Die bei anderen Methoden störenden Rezirkulationserscheinungen werden durch das automatische „Auslöschen" des Indikators beseitigt. Die Blutviskosität wird nicht beeinflusst.

Die beschriebene Methode erlaubt auch andere Messungen. So kann die Zeit Lunge-Ohr abgelesen werden, wenn der Anfang des ersten Atemzuges nach einer Veränderung des geatmeten Gasgemisches auf der Kurve markiert wird. Es ist zu erwarten, wenn das untersuchte Organ aus zwei Teilen mit stark von einander abweichenden Kreislaufzeiten besteht, dass beide Zeiten gemessen werden können, indem die Venenkurve zwei Minima bzw. einen treppenförmigen Anstieg aufweisen wird.

Nachteile der Methode. Die Messung der kürzesten Kreislaufzeit, die Zeit vom Erscheinen des Indikators an der einen Messstelle bis zum Erscheinen an der zweiten, ist insofern unvorteilhaft, als die Variationen der Zeitwerte klein werden und die erhaltenen Werte nicht zur Bestimmung vom Flüssigkeitsvolumen herangezogen werden können. Prinzipiell ist jedoch zu erwarten, dass die kürzeste Kreislaufzeit dieselbe Abhängigkeit vom Stromvolumen zeigen wird wie die mittlere, sofern die Struktur des untersuchten Systems gleich bleibt. Eigene Modellversuche mit von Blut durchströmten Glasgefässen in variierender Anordnung haben dies bestätigt und eine lineare Abhängigkeit der inversen Zeitwerte — als Mass für die lineare Strömungsgeschwindigkeit — vom Stromvolumen gezeigt. Die Schwierigkeit, die mittlere Kreislaufzeit anzugeben, hängt mit dem Sauerstoffverbrauch des Gewebes zusammen, der die Amplituden der Kurvenausschläge beeinflusst.

Für die Feststellung der kürzesten Kreislaufzeit, des Zeitpunktes des Erscheinens der Sättigungsveränderung, ist der Einfluss des Sauerstoffverbrauches dagegen nicht stark störend. Da grosse Änderungen des Sauerstoffverbrauches während einer Messung nicht zu erwarten sind, hat die Sättigungsmethode durch die ausserordentliche Kleinheit der physikalisch gelösten im Verhältnis zur hämoglobingebundenen Sauerstoffquantität, eher einen Vorzug gegen Methoden mit anderen leicht diffusiblen Gasen (N_2O, Kr) als Indikatoren. Bei diesen kann mit einer grossen Konzentrationsabnahme der ersten Portionen des Indikatorstoffes bei der Passage durch

das Gehirn gerechnet werden, wodurch ein zu hoher Wert für die kürzeste Kreislaufzeit resultiert.

Gewisse Schwierigkeiten bei der angegebenen Methode ergeben sich bei den Messungen am Venenblut. Die Entnahme aus der Vene führt zu einem Blutverlust von 5 ml pro Minute. Eine gewisse Aufmerksamkeit auf die Lage der Punktionskanüle in der Vene ist erforderlich, um Unterbrechungen in der Aspiration zu vermeiden. Dadurch, dass die Oxalatkammer erst hinter der Cuvetteneinheit eingeschaltet ist, entsteht in der Einheit eine gewisse Koagulationsgefahr. Diese kann durch Injektion von 50 mg Heparin vor der Untersuchung bedeutend vermindert werden. Im allgemeinen ist es auch ohne Heparin möglich, 10—15 Minuten ohne störende Gerinnung zu arbeiten.

Die an das Hämoglobin gebundene Messung betrifft naturgemäss nur die Erythrozyten, die wie bekannt eine höhere lineare Geschwindigkeit als das Plasma haben. Nach FREIS, STANTON und EMERSON (1949) gilt dies doch nicht für die kürzeste Kreislaufzeit (appearance time), die für Plasma und Blutkörperchen gleich ist. Diese Tatsache ist auch aus den Kurven von DOW, HAHN und HAMILTON (1946) ersichtlich.

Vergleich mit anderen Methoden. Ein direkter Vergleich mit den zahlreichen Kreislaufzeitmessungen, die mit röntgendiagnostischer Methode nach intraarterieller Injektion von Kontrastmitteln durchgeführt worden sind, lässt sich kaum durchführen. Diese Zeitwerte sind nach sehr verschiedenen Prinzipien abgemessen worden und betreffen im allgemeinen nur Teile der ganzen Organpassage; auch die Verschiedenheit der Registriertechnik erschwert die vergleichende Betrachtung. Schliesslich rechnet man damit, dass die Kreislaufverhältnisse durch die Messprozedur verändert werden in einem Ausmasse, das nach der jeweiligen Injektionstechnik variieren kann. Eine ausführliche Darstellung dieser Probleme findet sich bei GREITZ (1956).

Untersuchungen mittelst Injektion von radioaktiven Substanzen in die A. carotis und direkter Beobachtung der Radioaktivität des strömenden Blutes auf der venösen Seite wurden von WOLFF und BLUMGART (1929) und von GREITZ (1956) ausgeführt. WOLFF und BLUMGART bestimmten die kürzeste Kreislaufzeit Carotis — Herz bei Katzen zu 6.05 sec. GREITZ hat, wie auch wir, bei seinen Untersuchungen am Menschen nicht die Injektion als Ausgangspunkt gewählt, sondern er konstruiert aus einer Registrierung der Impulse in der Höhe

des Bulbus jugularis eine Kurve für den zeitlichen Verlauf der Aktivität in der A. carotis und in der V. jugularis. Er misst die Zeit vom Maximum in der Arterie zum Maximum in der Vene, also die „häufigste" Kreislaufzeit. Von diesem Unterschied abgesehen können seine Resultate mit denen der hier angegebenen Methode direkt verglichen werden.

Die Methode von NYLIN und BLÖMER (1955), bei der ein radioaktiver Indikator in eine A. carotis injiziert wird, ist nach dem Prinzip der gewöhnlichen Farbstoffverdünnungsmethode aufgebaut, d.h. die Injektion wird als Ausgangspunkt benutzt und eine Aktivitätskurve des Venenblutes durch Analysen einer grösseren Zahl nacheinander aus dem Bulbus jugularis entnommener Blutproben konstruiert. Hierdurch wird es möglich, die mittlere Kreislaufzeit zu bestimmen, ebenso unter gewissen Voraussetzungen die absolute Blutvolumdurchströmung. Die Bestimmung der kürzesten Kreislaufzeit wird wegen der diskontinuierlichen Probeentnahmen weniger exakt. (Vgl. S. 93.).

Eine entsprechende Untersuchung mit Injektion von Farbstoffen scheint bisher nicht unternommen worden zu sein.

Schliesslich kann die Methode des Einatmens eines indifferenten Gases, wie sie ursprünglich von KETY und SCHMIDT (1945) eingeführt wurde, auch zur Bestimmung von Kreislaufzeiten herangezogen werden, wenngleich bisher nur LASSEN und MUNCK (1955) solche Gesichtspunkte angelegt haben. Die Bedeutung der Gaskonzentrationen für die Analyse von Kreislaufzeiten kann aus den folgenden Überlegungen ermittelt werden.

Der Kety'schen Methode liegt die Gleichung

$$Q = F\,A \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

zu Grunde, wo Q die vom Gehirn bei eingetretenem Gleichgewicht (Zeitpunkt n) aufgenommene (absolute) Quantität des Indikatorgases, F die (absolute) Blutvolumdurchströmung und A die Area zwischen der arteriellen und der venösen Gaskonzentrationskurve bezeichnet [also $A = \int_0^n (C_A - C_V)\,dt$, wenn C_A und C_V die mit der Zeit variierende Konzentration des Indikators im arteriellen bzw. venösen Blut, t die Zeit angibt].

Die absolute Quantität des Indikators im Gehirn ist auch

$$Q = F\,C_e\,T_M \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Hier bezeichnet C_e die Konzentration des Gases im arteriellen und venösen Blut nach eingetretenem Aequilibrium, T_M die mittlere Kreislaufzeit des Indikators. Die Gleichung entspricht dem von STEWART (1921 b) eingeführten und von MEIER und ZIERLER (1954) mathematisch näher analysierten Ausdruck $V = F\,T_M$. Dem Flüssigkeitsvolumen V entspricht die totale Gasquantität Q; die in der Zeiteinheit durschströmende Gasquantität ist $F\,C_e$.

Aus Gleichung (1) und (2) ergibt sich $F\,A = F\,C_e\,T_M$

d.h.
$$T_M = \frac{A}{C_e}.$$

Für die Bestimmung von Flüssigkeitsvolumina folgt hieraus

$$V = \frac{F\,A}{C_e}.$$

Diese Formel, auf andere Weise eruiert, ist von BRADLEY und Mitarb. 1953 für Volumenmessung angegeben, ihre Brauchbarkeit von RABINOWITZ und RAPAPORT (1954) nochmals hervorgehoben. Diese Methode enthält also indirekt auch eine Bestimmung der mittleren Kreislaufzeit.

Da man bei der Methode nach KETY die cerebrale Blutdurchströmung pro 100 g Gehirn

$$CBF = \frac{100\,C_e\,S}{A}$$

hat, folgt

$$T_M = \frac{100\,S}{CBF}.$$

S ist der Verteilungskoeffizient des Gases zwischen Gehirn und Blut.

Die Normalwerte für CBF liegen in der Grössenordnung 50—65 ml Blut/100 g · min, woraus sich bei $S = 1.0$ à 1.1 der Normalwert 1.5—2.2 Minuten für die mittlere Kreislaufzeit des Indikatorgases zwischen den beiden Messpunkten ergibt. Die mittlere Kreislaufzeit des Blutes wird sich mit dieser Methode nicht feststellen lassen, da der Indikator sich auch ausserhalb des Blutes verteilt. Die von LASSEN und MUNCK (1955) durch rückwärtige Extrapolation der Anfangsteile der Konzentrationskurven ermittelte Messung betrifft die Zeit vom arteriellen zum venösen Erscheinen des Indikators. Bei dieser kürzesten Kreislaufzeit wäre keine nennenswerte Abweichung zwischen den Zeiten des indifferenten Gases und der Blutkörperchen zu erwarten. Da aber, wie früher ausgeführt, die Diffusion in das Gewebe einen Einfluss auf die Zeitmessung

haben kann, besteht eine gewisse Wahrscheinlichkeit dafür, dass diese Methode zu hohe Werte für die kürzeste Kreislaufzeit geben wird.

Es können demnach gegen sämtliche bisher vorgeschlagene Methoden zur Messung cerebraler Kreislaufzeiten Einwände erhoben werden. Die hier beschriebene Methode der Sättigungsmessung hat im Vergleich mit anderen Verfahren den Vorteil der schnellen Wiederholbarkeit der Messungen. Sie ist ausserdem die einzige, bei der eine Arterienpunktion vermieden werden kann, eine Tatsache, die durch die in dieser Arbeit angeführten Bedenken gegen intraarterielle Injektionen an Bedeutung zunimmt.

Zusammenfassung.

Eine Methode zur Messung der Kreislaufzeit durch das menschliche Gehirn unter Verwendung von zwei Oximetereinheiten wird beschrieben.

Die Sauerstoffsättigung des arterialisierten Ohrblutes und die des venösen Blutes im Bulbus jugularis werden gleichzeitig registriert mit Hilfe einer Ohreinheit bzw. einer Cuvetteneinheit, die auf der Punktionskanüle des Bulbus jugularis montiert ist. Das Jugularisblut wird von einer Rekordspritze mit Synchronmotorantrieb gleichmässig durch die Einheit gesogen.

Durch kurzdauerndes Stickstoffatmen werden rasche Änderungen der Sättigung des Blutes hervorgerufen. Die kürzeste Kreislaufzeit wird als Abstand zwischen korrespondierenden Punkten der Arterien- und Venenkurve gemessen.

Nach Injektionen von NaCl oder EVANS blue in die A. carotis communis wurden Befunde erhoben, die darauf deuten, dass die Gehirndurchblutung unmittelbar nach einer intraarteriellen Injektion vorübergehend zunimmt.

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